

## CHAPTER 3

### RESULTS AND DISCUSSION

#### 1. Characterization of black tiger shrimp and white shrimp meats

##### 1.1 Proximate composition

Black tiger shrimp and white shrimp meats had the moisture content of 80.47 and 77.21%, respectively. Protein was found as the major constituent, indicating that shrimp meat can be the good source of amino acids. Black tiger shrimp meat comprised higher moisture content (Table 5) but lower protein content than did white shrimp meat ( $p < 0.05$ ). No differences in fat content between two species were observed ( $p > 0.05$ ). A greater ash content was noticeable in white shrimp meat, compared with that of black tiger shrimp meat. Proximate compositions of the edible part of red shrimp, pink shrimp and lobster were slightly different (Rosa and Nunes, 2003). Differences in proximate composition might result in the differences in nutritional value, sensory qualities and shelf-life of both shrimps. Proximate compositions in shrimp muscles are governed by many factors including species, growth stage, feed and season (Sikorski *et al.*, 1990; Karakoltsidis *et al.*, 1995).

Table 5 Proximate composition of black tiger shrimp and white shrimp meats

Compositions (% wet wt. )	Black tiger shrimp	White shrimp
Moisture	$80.47 \pm 0.26^a$	$77.21 \pm 0.18^b$
Ash	$0.95 \pm 0.01^b$	$1.47 \pm 0.10^a$
Protein	$17.09 \pm 0.56^b$	$18.75 \pm 0.23^a$
Fat	$1.23 \pm 0.36^a$	$1.30 \pm 0.09^a$

Values are given as mean  $\pm$ SD from triplicate determinations. Different superscripts in the same row indicate significant differences ( $p < 0.05$ ).

## 1.2 Proteins and non-protein nitrogenous compounds

Proteins and non-protein nitrogenous components in both black tiger shrimp and white shrimp meats are shown in Table 6. Myofibrillar protein constituted as the major protein in both shrimps. Sarcoplasmic protein was found as the second predominant protein in the shrimp meats. The result was in agreement with Hashimoto *et al.* (1979) who reported that myofibrillar and sarcoplasmic proteins are the major proteins in fish muscle. From the result, white shrimp had the higher myofibrillar protein, sarcoplasmic protein and stromal proteins than did black tiger shrimp ( $p < 0.05$ ). However, a lower alkali-soluble protein content was found in white shrimp, compared with black tiger shrimp ( $p < 0.05$ ). The major fraction of stromal protein is collagen (Foegeding *et al.*, 1986). The differences in protein compositions, especially stromal protein might determine the quality attributes of both species. When considering the non-protein nitrogenous components between both species, it was noted that black tiger shrimp had a greater content than did white shrimp ( $p < 0.05$ ). Such a difference might affect the flavor and taste of both shrimps. Non-protein nitrogenous constituents such as free amino acids, peptide, betaine and nucleotide play an essential role in flavor of fish and shellfish (Sikorski *et al.*, 1990). Generally, black tiger shrimp has been recognized to possess the superior acceptability in term of flavor and taste to white shrimp. This might be governed by the different amount and compositions of non-protein nitrogenous constituents.

Table 6 Nitrogenous constituents in black tiger shrimp and white shrimp meats

Composition (mg N/g muscle)	Black tiger shrimp	White shrimp
Non-protein nitrogen	4.68 ± 0.31 <sup>a</sup>	1.44 ± 0.23 <sup>b</sup>
Sarcoplasmic protein	6.16 ± 0.02 <sup>b</sup>	7.81 ± 0.62 <sup>a</sup>
Myofibrillar protein	12.62 ± 0.35 <sup>a</sup>	14.25 ± 0.99 <sup>a</sup>
Alkali-soluble protein	0.65 ± 0.05 <sup>a</sup>	0.36 ± 0.03 <sup>b</sup>
Stromal protein	0.21 ± 0.01 <sup>b</sup>	2.66 ± 0.11 <sup>a</sup>

Values are given as mean ±SD from triplicate determinations. Different superscripts in the same row indicate significant differences ( $p < 0.05$ ).

SDS-PAGE patterns of whole meat from black tiger shrimp and white shrimp meats are shown in Figure 7. Both shrimps had myosin heavy chain (MHC) as the dominant protein component, constituting around 56.81-64.25% of total proteins. MHC is the major protein in myofibrillar protein (Shahidi, 1994). Actin was found as the second dominant protein. In general, similar protein patterns were noticeable between both shrimps. Nevertheless, slight differences in band intensity of proteins with MW of 84 and 66 kDa were observed. Protein patterns of different fractions of both shrimps are illustrated in Figure 8. For myofibrillar protein fraction, two major protein bands, corresponding to MHC and actin, were observed. Generally, low MW proteins were found in the sarcoplasmic protein fraction. Protein with MW of 24 kDa was predominant. For alkali fraction, smear bands with low MW were found in black tiger shrimp. For alkali fraction of white shrimp, proteins with MW of 36, 29 and 24 kDa constituted as the major protein. Similar protein pattern was noticeable between stromal fraction of both species. The differences in protein compositions might result in the different properties and characteristics between both shrimps.

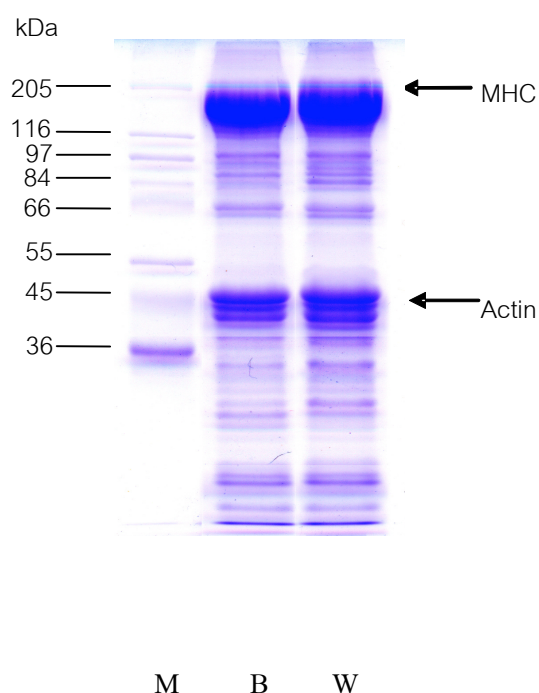


Figure 7 Electrophoretic pattern of black tiger shrimp and white shrimp meats. M: high-molecular-weight protein marker, B: black tiger shrimp meat, W: white shrimp meat.

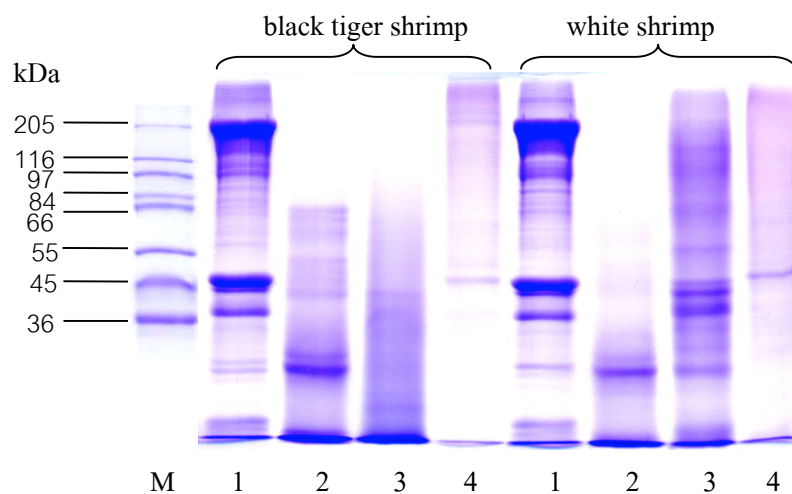


Figure 8 Electrophoretic pattern of various protein fractions from black tiger shrimp and white shrimp meats. lanes 1, 2, 3, 4: myofibrillar, sarcoplasmic, alkali-soluble and stromal protein fractions of black tiger shrimp meat, respectively.

### 1.3 Collagen

PSC and ISC from black tiger shrimp and white shrimp meats were isolated with the yield of 0.36, 0.48% and 0.83, 3.32% (dry weight), respectively. From the results, white shrimp meat contained the higher PSC and ISC than did black tiger shrimp meat. The higher collagen content observed in white shrimp meat was coincidental with the greater stromal protein content (Table 6). Yoshinaka *et al.* (1989) reported that collagen content in the abdominal muscle of seven species including shrimp, prawn, lobster and squilla varied among the species ranging from 1.1 to 6.2% of total tissue protein and the content in pereiopod and thoracic muscles of four species of crab varied from 0.2 to 0.8%. The higher collagen content was associated with the firmer texture (Sato *et al.*, 1986; Hatae *et al.*, 1986).

The protein patterns of PSC and ISC of both shrimp meats are shown in Figure 9. PSC from both species had the patterns similar to those of porcine cartilage collagen type A and AI (Figure 9). Type A collagen consists of 3 chains of  $\alpha 1(A)$  and Type AI collagen consists of 3 chains of  $\alpha 1(AI)$  (Foegeding *et al.*, 1986). Yoshinaka *et al.* (1989) reported that the major collagen from the crustacean muscle was similar to type V collagen from the vertebrate muscle. However, the type of collagen of fish was different depending upon species. For ISC, different protein patterns were found between both shrimps. ISC was most likely associated with the cross-

linking of collagen molecules, which was postulated to have the influence on the texture of shrimp meat. Few cross-links indicate a low shear strength (Montero and Borderias, 1990). The gaping score in fish correlated with the amount of insoluble collagen; the more ISC, the less gaping was found (Espe *et al.*, 2004). The distribution of soluble and insoluble collagen in fish muscle varied from species to species (Eckhoff *et al.*, 1998).

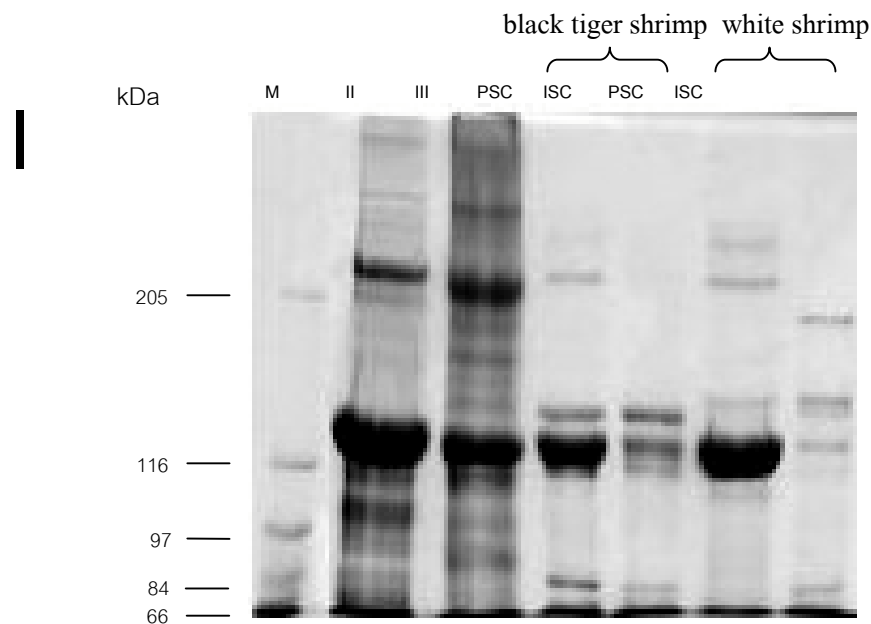


Figure 9 Electrophoretic pattern of PSC and ISC from black tiger shrimp and white shrimp meats. lanes 1,2: porcine cartilage collagen type A and AI, respectively. PSC: pepsin soluble collagen; ISC: insoluble collagen.

#### 1.4 Amino acid compositions

The amino acid compositions of black tiger shrimp and white shrimp meats are shown in Table 7. Amino acids from both shrimps were different between species. Yanar and Celik (2006) also reported the different amino acid compositions between green tiger shrimp (*Penaeus semisulcatus* De Haan, 1844) and speckled shrimp (*Metapenaeus monoceros* Fabricus, 1789). From the results, the most abundant amino acid in both species was arginine. High content of free arginine in crustaceans enriches the sweet taste and yields a seafood-like flavor (Sikorski *et al.*, 1990). Proline, leucine, isoleucine, phenylalanine and glutamic acid were found

predominant in both species. These amino acids constituted more than 50% of the total amino acids. Glycine, alanine, serine and threonine taste sweet, while arginine, leucine, valine, methionine, phenylalanine, histidine and isoleucine give bitter taste (Sikorski *et al.*, 1990). Alanine, proline and serine contributed to the acceptability of prawns and lobsters (Fuke, 1994). A higher glycine content was found in black tiger shrimp (3.10%), compared with white shrimp (2.99%). The sweetness of fresh prawn and crab is attributed to the abundance of free glycine in their muscle (Sikorski *et al.*, 1990). From the result, black tiger shrimp meat contained the higher contents of arginine, phenylalanine, isoleucine, glutamic acid, but comprised the lower contents of aspartic acid, proline and leucine than did white shrimp meat. Apart from amino acids, the nucleotides and quaternary ammonium compounds are the major contributors to the taste of seafoods (Sikorski *et al.*, 1990). AMP and ATP are the dominating nucleotides in crustaceans and mollusks, immediately post mortem (Mendes *et al.*, 2001).

Black tiger shrimp and white shrimp contained 9.70% and 13.26% of proline, respectively. Proline showed an important adjustment necessary for osmoregulation, following changes in osmotic stress (Bishop and Burton, 1993). Hydroxyproline content was higher in white shrimp meat, compared with black tiger shrimp meat. This was coincidental with the higher stromal protein content in the former (Table 6). The ratios of essential amino acids (EAA) to nonessential amino acids (NEAA) in black tiger shrimp and white shrimp were 0.70 and 0.67, respectively. Iwasaki and Harada (1985) explained that EAA/NEAA ratio of many fish species is 0.70 on average, whereas this ratio was reported to be 0.59 in crab (*Portunus trituberculatus*) and squid (*Doryteuthis bleekeri*). Thus, the different amino acids might be associated with the varying taste as well as textural properties of meat of both shrimps.

Table 7 Amino acid compositions of black tiger shrimp and white shrimp meats (mg/100 g)

Amino acids	Black tiger shrimp	White shrimp
Aspartic acid + Asparagine	1456.1	1704.0
Hydroxyproline	69.8	214.6
Threonine	1213.3	1128.5
Serine	1068.7	1026.7
Glutamic acid + Glutamine	1854.0	1503.5
Proline	2889.2	3861.7
Glycine	1181.6	870.5
Alanine	1524.5	1601.6
Cysteine	528.4	546.8
Valine	1158.6	1077.9
Methionine	1395.7	1297.6
Isoleucine	2585.8	2410.8
Leucine	2974.3	3152.7
Tyrosine	1955.5	1967.2
Phenylalanine	2277.0	1967.2
Hydroxylysine	82.4	-
Lysine	653.5	629.6
Histidine	666.8	665.9
Arginine	4272.9	3494.0
Total	29808.1	29120.8

### 1.5 Lipid composition and fatty acid profile of shrimp meat

Lipids from both shrimp meats were composed of phospholipids as the major component (72-74%), followed by triglyceride (Table 8). Therefore, most lipids in shrimp meat might be membrane lipids with high phospholipid content. Takama *et al.* (1999) reported that phospholipids were the major lipids in fish muscle. The higher free fatty acid content with coincidental lower diglyceride content in white shrimp, compared with black tiger shrimp,

suggested that lipid from white shrimp was more susceptible to hydrolysis caused by lipase. Lipase and phospholipase in shrimp muscle played an important role in hydrolysis of lipids (Lopez and Marangoni, 2000).

Table 8 Lipid composition of black tiger shrimp and white shrimp meats

Compositions (% dry wt.)	Black tiger shrimp	White shrimp
Phospholipid	74.53 ± 0.02 <sup>a</sup>	72.28 ± 2.27 <sup>a</sup>
Triglyceride	16.29 ± 0.01 <sup>a</sup>	16.22 ± 0.06 <sup>a</sup>
Diglyceride	6.70 ± 0.29 <sup>a</sup>	2.71 ± 0.01 <sup>b</sup>
Free fatty acid	2.46 ± 0.16 <sup>b</sup>	8.77 ± 0.07 <sup>a</sup>

Values are given as mean ±SD from triplicate determinations. Different superscripts in the same row indicate significant differences. (p<0.05)

The fatty acid profiles of black tiger shrimp and white shrimp meats are shown in Table 9. PUFAs were found as the major fatty acids with the range of 42.20-44.43%. The result was in agreement with Lin *et al.* (2003) who found that PUFAs were the major fatty acids in white shrimp. The contents of n-3 PUFA in both shrimps were 1.14-fold greater than those of n-6 PUFA. C22:6 n-3(DHA) and 20:5 n-3(EPA) were the dominant PUFAs in lipid from both shrimps. DHA and EPA were found at the level of 14.88 and 8.58% in the lipid from black tiger shrimp and 9.99 and 9.46% in the lipid from white shrimp. DHA/EPA ratio in black tiger shrimp meat (2.15) was higher than that found in white shrimp meat (1.05). White shrimp meat comprised higher oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) than did black tiger shrimp meat. Among the saturated fatty acids, C16:0 and C18:0 were the most abundant fatty acids in the lipid extracted from black tiger shrimp and white shrimp. Rosa and Nunes (2003) and Yanar and Celik (2005) reported that palmitic acid (C16:0), stearic acid (C18:0), DHA and EPA were found to be the most abundant fatty acids in shrimps (*Nephrops norvegicus*, *Parapenaeus longirostris*, *Aristeus antennatus* and *Penaeus semisulcatus*, *Metapenaeus monoceros*).



Table 9 Fatty acid composition (g/100g) of black tiger shrimp and white shrimp meats

Fatty acids	black tiger shrimp	white shrimp
Myristic acid C14:0	0.38	0.41
Pentadecanoic acid C15:0	0.38	0.37
Palmitic acid C16:0	22.15	21.80
Palmitoleic acid C16:1 n-7	1.42	1.39
Heptadecanoic acid C17:0	1.53	1.45
Cis-10-Heptadecenoic acid C17:1	0.31	0.24
Stearic acid C18:0	10.51	11.50
Oleic acid C18:1 n-9	9.94	11.37
Cis-Vaccenic acid C18:1 n-7	2.18	2.41
Linoleic acid C18:2 n-6	12.95	15.62
□ -Linolenic acid C18:3 n-3	0.77	0.98
Υ-Linolenic acid C18:3 n-6	0.25	0.30
Arachidic acid C20:0	0.15	0.23
Cis-11-Eicosenoic acid C20:1 n-9	0.4	0.53
Cis-11-Eicosenoic acid C20:1 n-11	0.18	0.16
Cis-11,14-Eicosadienoic acid C20:2 n-6	0.68	1.40
Cis-11,14,17-Eicosatrienoic acid C20:3 n-3	0.12	0.18
Arachidonic acid C20:4 n-6	4.55	3.23
Eicosatetraenoic acid C20:4 n-3	0.15	-
Cis-5,8,11,14,17-Eicosapentaenoic acid C20:5 n-3 (EPA)	8.58	9.46
Behenic acid C22:0	0.18	-
Docosatetraenoic acid C22:4 n-6	0.21	0.20
Docosapentaenoic acid C22:5 n-3	0.60	0.50
Docosapentaenoic acid C22:5 n-6	0.69	0.34
Cis-4,7,10,13,16,19-Docosahexaenoic acid C22-6 n-3 (DHA)	14.88	9.99
Lignoceric acid C24:0	0.14	-
Nervonic acid C24:1	0.22	-
Unidentified peak	5.50	5.94
PUFA	44.33	42.20
n3 PUFA	25.10	21.11
n6 PUFA	19.33	21.09
SFA	35.42	35.76

PUFA: polyunsaturated fatty acids; SFA: saturated fatty acid

### 1.6 Mineral content of shrimp meat

The contents of different minerals in both shrimp meats, black tiger shrimp and white shrimp are shown in Table 10. Black tiger shrimp meat contained the higher content of all minerals determined than did white shrimp meat. From the result, Mg was the dominant mineral in both shrimp meats. Ca and Fe were also found at the high content. Ca is essential for hard tissue structure, blood clotting, muscle contraction, nerve transmission, osmoregulation and as a cofactor for enzymatic procession (Lovell, 1989). Transition metal ions, particularly Cu and Fe, have been known as the major catalysts for oxidation (Thanonkaew *et al.*, 2006). Cu ion is found in hemocyanin, a pigment in blood of crustacean (Decker and Tuczec, 2000). Those minerals might contribute to oxidation of shrimp muscle during handling, processing as well as storage. Castell *et al.* (1965) found that the relative prooxidant activity of ions in fish muscle decreased in the order of  $\text{Cu}^{2+} > \text{Fe}^{2+} > \text{Co}^{2+} > \text{Cd}^{2+} > \text{Li} > \text{Ni}^{2+} > \text{Mg}^{2+} > \text{Zn}^{2+} > \text{Ca}^{2+} > \text{Ba}^{2+}$ . Owing to a higher content of metal ions, black tiger shrimp meat might be more susceptible to lipid oxidation than white shrimp. Additionally, higher content of PUFAs in black tiger shrimp, especially DHA, might cause the meat more prone to oxidation. Major sources of minerals to marine organisms are sea water and feed (Ichihashi *et al.*, 2001). No cadmium and cobalt were detectable in both shrimp meats.

Table 10 Mineral contents in black tiger shrimp and white shrimp meats (mg/kg)

Minerals	Black tiger shrimp	White shrimp
Fe	30.65±0.19	12.24±0.42
Cu	6.31±0.02	4.07±0.16
Mn	1.00±0.00	0.48±0.00
Cd	ND	ND
Ni	0.60±0.02	0.36±0.01
Zn	17.33±0.09	14.65±0.56
Co	ND	ND
Ca	259.11±0.62	246.88±4.99
Mg	430.89±3.10	360.55±8.15

Values are given as mean ±SD from triplicate determinations. ND: not detectable.

## 2. Effect of heat treatment on physicochemical, physical properties and microstructure of black tiger shrimp and white shrimp meats

### 2.1 Thermal properties of muscle proteins

#### 2.1.1 Differential scanning calorimetry (DSC)

Thermal transitions of shrimps muscle proteins were determined using DSC.  $T_{max}$  and  $\Delta H$  are shown in Table 11. DSC analysis was used to determine the thermal transition or unfolding temperature of protein and also to quantify the enthalpy of conformational transition (John and Shastri, 1998). Two major peaks were obtained, corresponding to myosin and actin peaks. Both shrimps had similar  $T_{max}$  and enthalpy of the first peak, suggesting that myosin of both shrimp muscles had the similar temperature and energy required for denaturation. However,  $T_{max}$  of the second peak representing actin of black tiger shrimp was lower than that of white shrimp. However, no differences in enthalpy of actin were found between two species ( $p > 0.05$ ). Poulter *et al.* (1985) reported that  $T_{max}$  of the first and the second peak of fish were 41.70-52.70 and 72.6-73.8°C, respectively. The result revealed that actin of white shrimp meat was more likely stable to thermal denaturation, compare with that of black tiger shrimp meat.

Table 11  $T_{max}$  and enthalpy of muscle proteins of black tiger shrimp and white shrimp meats

Species	$T_{max}$ I (°C)	$\Delta H$ (J/g)	$T_{max}$ A (°C)	$\Delta H$ (J/g)
Black Tiger shrimp	51.28 ± 0.56 <sup>a</sup>	1.46 ± 0.06 <sup>a</sup>	66.20 ± 0.28 <sup>b</sup>	0.66 ± 0.07 <sup>a</sup>
White shrimp	50.13 ± 0.14 <sup>a</sup>	1.40 ± 0.03 <sup>a</sup>	71.17 ± 0.34 <sup>a</sup>	0.67 ± 0.10 <sup>a</sup>

Values are given as mean ±SD from triplicate determinations. Different superscripts in the same column indicate significant differences. ( $p < 0.05$ ).

#### 2.1.2 Thermal stability

The inactivation rate constants ( $K_D$  value) of natural actomyosin (NAM) from both shrimps are shown in Table 12. Slight increases in  $K_D$  values were noticeable at temperature below 20°C. At temperature ranges of 30-40°C, substantial increases in  $K_D$  value were observed. At the same temperature, NAM from white shrimp had slightly higher  $K_D$  value, compared to that from black tiger shrimp. From the results, it was presumed that muscle proteins, particularly MHC, of white shrimp were more susceptible to thermal denaturation than those of black tiger

shrimp. MHC has been reported to possess  $\text{Ca}^{2+}$ -ATPase activity, which can be used as the indicator of MHC integrity (Benjakul *et al.*, 1997). Actin was suggested to play a protective role in the stability of myosin (Jiang *et al.*, 1989). Thus, the stability of muscle protein from black tiger shrimp was slightly higher than that from white shrimp. The differences in thermal stability between both species possibly resulted from the different intrinsic properties, amino acid composition as well as actin/myosin ratio.

Table 12 Thermal inactivation rate constant ( $K_D \times 10^{-5} \text{ S}^{-1}$ ) of natural actomyosin from black tiger shrimp and white shrimp meats

Species	Temperature ( $^{\circ}\text{C}$ )				
	0	10	20	30	40
Black tiger shrimp	0.09±0.01 <sup>aE</sup>	3.14±0.22 <sup>aD</sup>	5.48±0.39 <sup>bC</sup>	40.87±2.31 <sup>aB</sup>	59.10±0.28 <sup>aA</sup>
White shrimp	0.10±0.02 <sup>aE</sup>	3.21±0.74 <sup>aD</sup>	8.46±1.86 <sup>aC</sup>	42.50±1.45 <sup>aB</sup>	62.45±2.24 <sup>aA</sup>

Values are given as mean  $\pm$ SD from triplicate determinations. Different superscripts in the same column indicate significant differences. ( $p < 0.05$ ). Different capital superscripts in the same row indicate significant differences. ( $p < 0.05$ ).

## 2.2 Effect of heat treatment on physical properties of shrimp meats

### 2.2.1 Changes in cooking loss of black tiger shrimp and white shrimp meats with different parts subjected to heat treatment

Cooking losses of black tiger shrimp and white shrimp meats with different parts (front, middle and tail) subjected to heating at  $100^{\circ}\text{C}$  for different times are shown in Figure 10A and 10B, respectively. No differences in cooking loss were found in all parts of both species when heated up to 1 min. Cooking loss of shrimp meats increased as heating time was above 1 min ( $p < 0.05$ ). With increasing core temperatures, the water content of meat decreased, while the fat and protein contents increased, indicating that the main part of the cooking loss is water (Heymann *et al.*, 1990). The cooking loss of all samples increased sharply when the samples were heated for more than one min ( $p < 0.05$ ). The water is probably lost due to heat induced denaturation of proteins during cooking of the meat, which causes less water to be entrapped within the protein structures held by capillary forces (Aaslyng *et al.*, 2003). The amount of denaturated proteins

depends not only on the center temperature but also on the holding time of each temperature (Martens *et al.*, 1982). Among all parts of shrimp meat, tail part of both shrimps had the highest cooking loss, particularly when heating time was greater than 1 min. Front part and middle part of both species showed the similar cooking loss when heated for 2-3 min ( $p>0.05$ ). The highest cooking loss of tail part of both shrimp meats might be associated with the highest surface area of this part, leading to the more release of water during cooking.

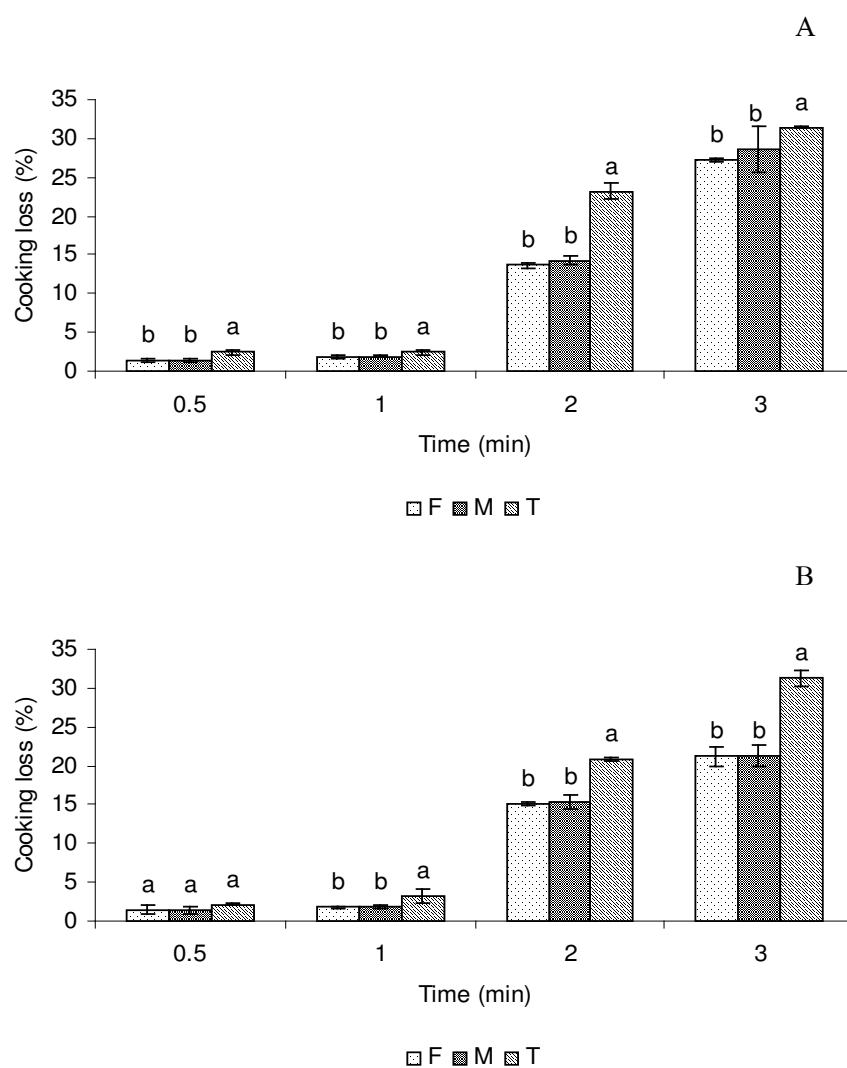


Figure 10 The influence of heat treatment (100°C) for different times on cooking loss of black tiger shrimp (A) and white shrimp meats (B); F: front; M: middle and T: tail. The same letters within the same part indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.

### **2.2.2 Changes in shear force of black tiger shrimp and white shrimp meats with different parts subjected to heat treatment**

Shear force of meats with different parts of black tiger shrimp and white shrimp subjected to heating at 100°C for different times is shown in Figure 11A and 11B, respectively. Shear force of shrimp meats increased as heating time increased. The shear force of all samples increased markedly when the samples were heated for more than 0.5 min ( $p < 0.05$ ). However, no differences in shear force were observed in all parts of black tiger shrimp when heated for 2-3 min ( $p > 0.05$ ). Shrimp meat is enhanced in firmness or solidity by heat processing and gets too solid and unpalatable when its inner temperature is above 100°C (Mizuta *et al.*, 1999). For the same heating time, tail part of both shrimps had the lowest shear force ( $p < 0.05$ ). Shear force correlated either with the diameter (width) of the muscle portion sheared or with the weight of the shrimp (Srinivasan *et al.*, 1997). The correlation of shrimp size with shear force indicated that the size of muscle or bundles had a major effect on meat tenderness (Srinivasan, *et al.*, 1997). From the result, black tiger shrimp and white shrimp had the slight differences in shear force either before or after heating. Due to the same size of shrimp used, the differences in composition and microstructure and the arrangement of muscle fiber between both species were postulated. Heating might cause the shrinkage of muscle fibers and losses in water held in the muscle structure. This might contribute to the increased shear force of heated samples.

### **2.2.3 Changes in color of black tiger shrimp and white shrimp meats with different parts subjected to heat treatment**

The colors ( $L^*$ ,  $a^*$  and  $b^*$ -value) of black tiger and white shrimp meats with different parts heated at 100°C for different times are shown in Figure 12 and Figure 13, respectively. The differences in  $L^*$ ,  $a^*$ , and  $b^*$ -values were observed between species, suggesting the differences in pigments. However, the similar values were observed with different parts of the same species.  $L^*$ -value increased when heating time increased ( $p < 0.05$ ).  $L^*$ -values of all samples increased sharply when the samples were heated for more than 0.5 min ( $p < 0.05$ ). The  $a^*$  and  $b^*$ -values of all samples also increased when heating time increased ( $p < 0.05$ ). For both shrimps, no differences in  $L^*$ ,  $a^*$  and  $b^*$ -values were observed when heating time was more than 1 min ( $p > 0.05$ ). Nevertheless,  $a^*$  and  $b^*$ -values of white shrimp still increased as heating time increased up to 3 min ( $p < 0.05$ ). The results suggested that heating caused the denaturation of muscle proteins

as well as carotenoproteins. As a consequence, the carotenoids became dominant as shown by the increase in both  $a^*$  and  $b^*$ -values. Generally, black tiger shrimp had higher  $a^*$ -value than white shrimp. From the result, black tiger shrimp might have higher carotenoid content than white shrimp. The main pigment of shrimps is astaxanthin, a carotenoid pigment commonly found in crustacean (Yanar *et al.*, 2004). It provides the tissue with red–orange pigmentation (Okada *et al.*, 1994). Moreover, both shrimps had slight differences in  $b^*$ -value. The color of shrimp was different, depending upon species, feed, season and environment (Yanar *et al.*, 2004).

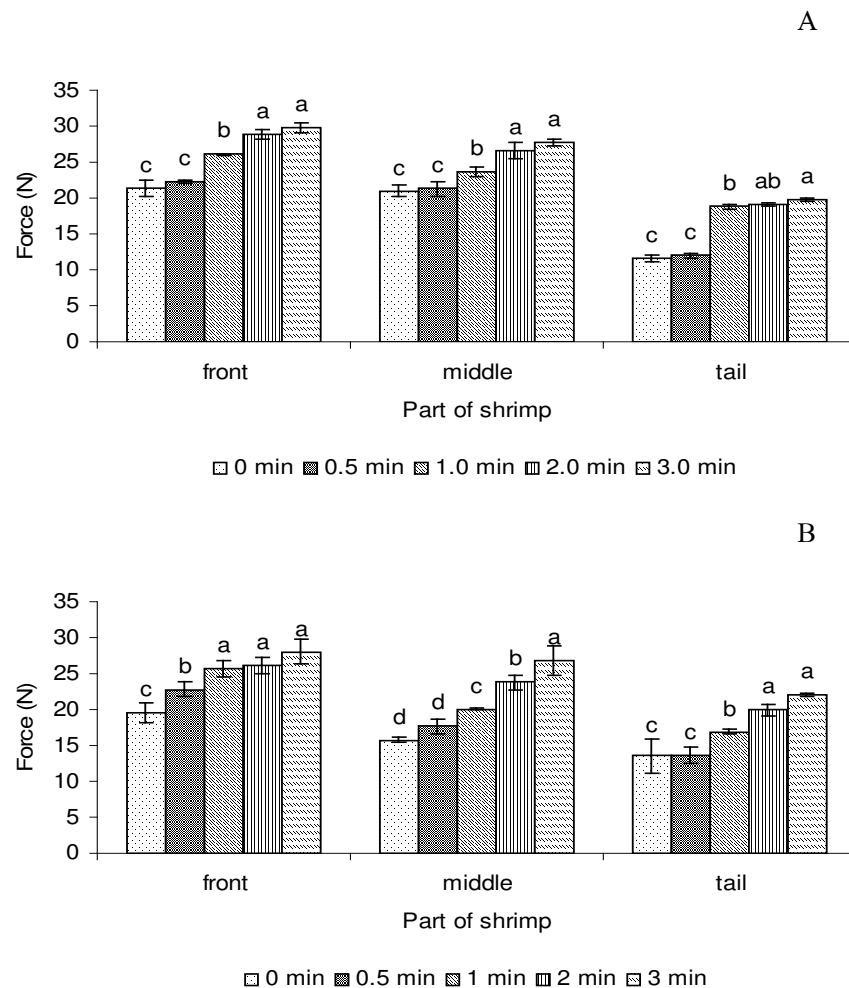


Figure 11 The influence of heat treatment ( $100^{\circ}\text{C}$ ) for different times on shear force of black tiger shrimp (A) and white shrimp meats (B); F: front; M: middle and T: tail. The same letters within the same part indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.

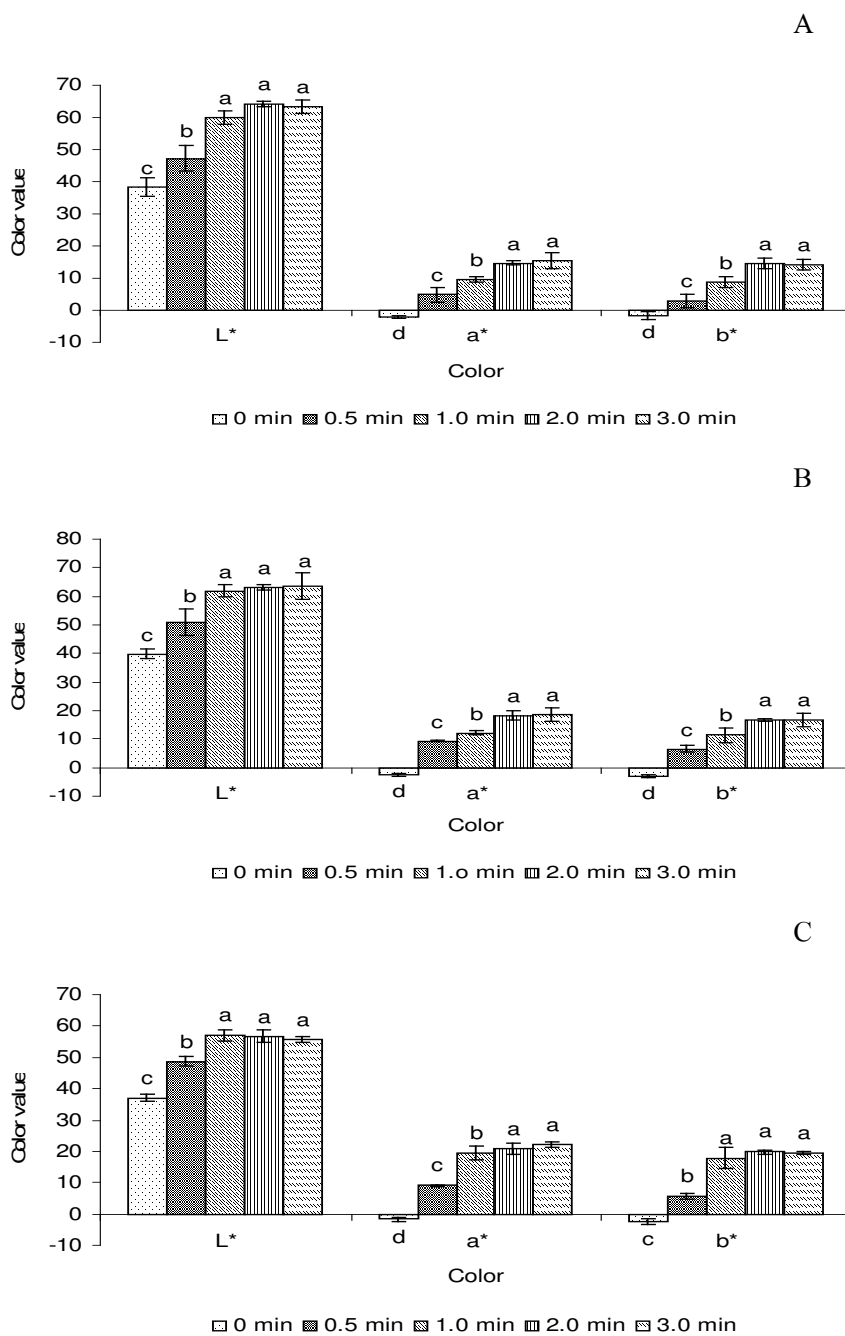


Figure 12 The influence of heat treatment (100°C) for different times on color of black tiger shrimp; A: front; B: middle and C: tail. The same letters within the same color parameter indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.



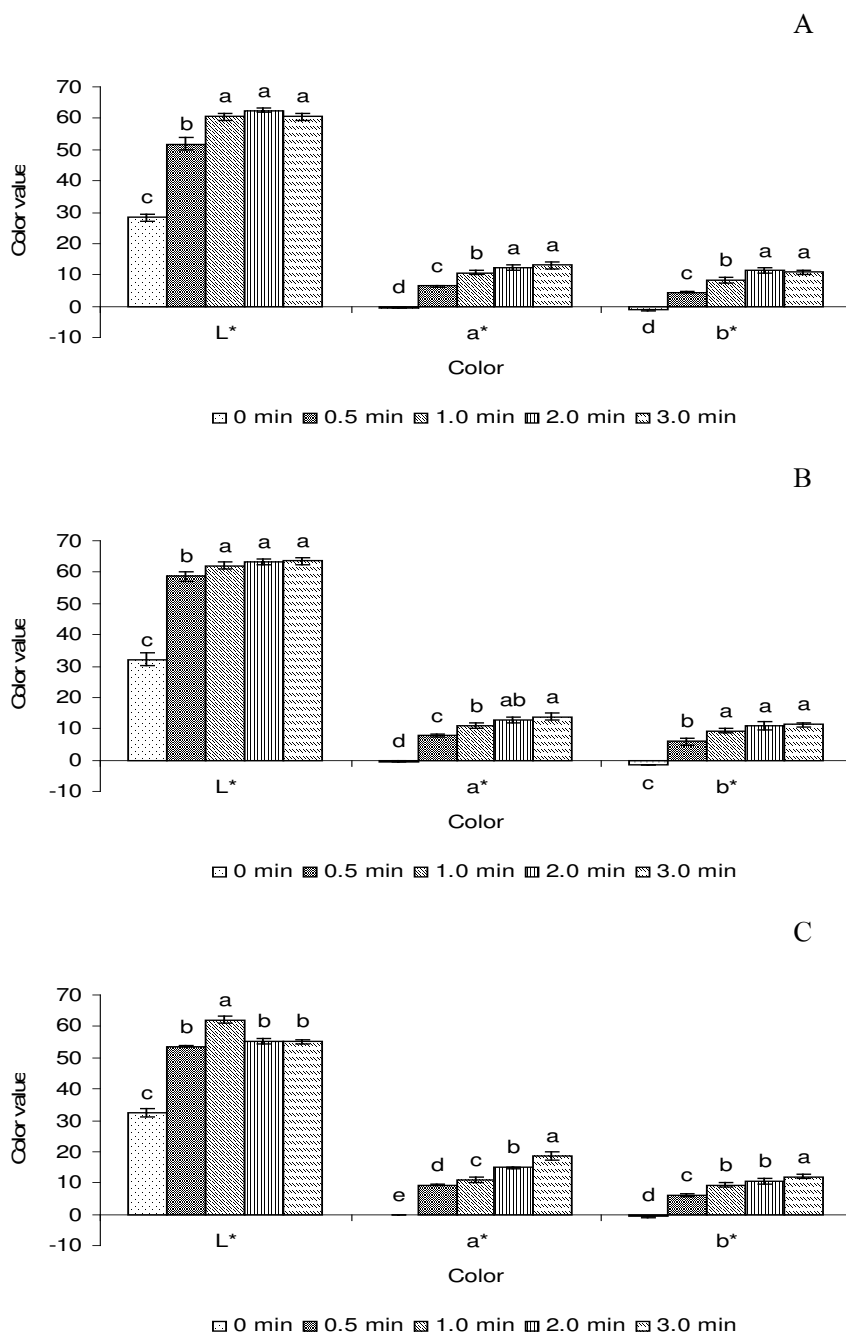


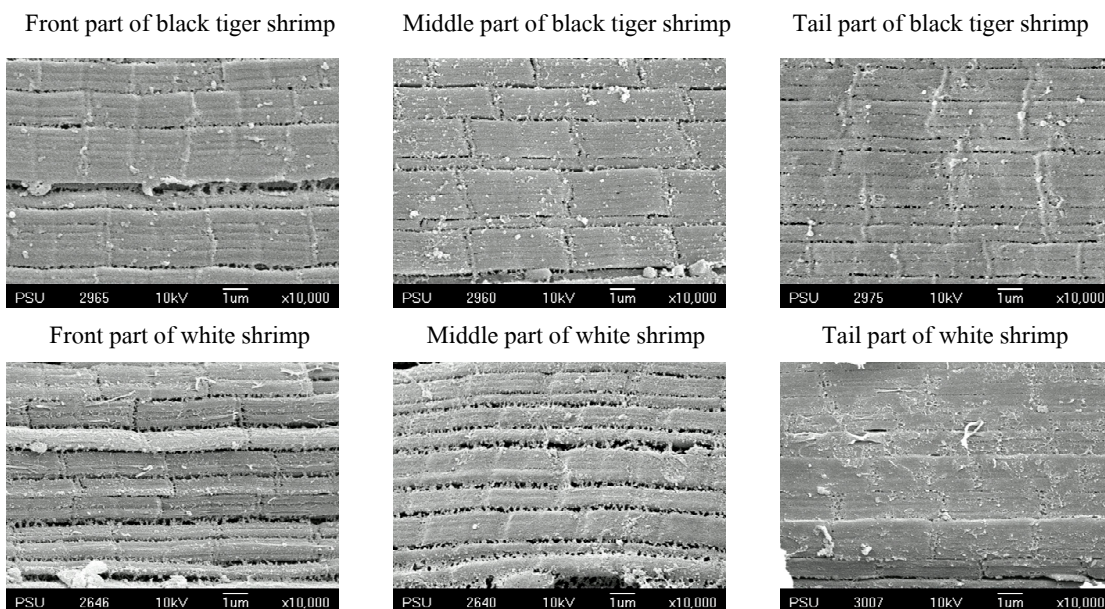
Figure 13 The influence of heat treatment (100 °C) for different times on color of white shrimp; A: front; B: middle and C: tail. The same letters within the same color parameter indicate non significant differences ( $p > 0.05$ ). Bars represent the standard deviation from triplicate determinations.

### 2.3 Changes in microstructure of raw and cooked black tiger shrimp and white shrimp meats with different parts

Microstructures of raw and cooked black tiger shrimp and white shrimp meats from different parts (front, middle and tail) are presented in Figure 14 and 15, respectively. For the longitudinal section, raw meats showed the slight differences in term of sarcomere length and density of fiber bundle between both species (Figure 14A). Among three parts of meats, tail portion tended to have the denser structure, compared to others. The marked changes in microstructure of black tiger shrimp and white shrimp were observed after heating (Figure 14B). The shrinkage of sarcomere was obvious in cooked shrimps of both species. It was suggested that heating process caused the shrinkage of muscle of black tiger shrimp and white shrimp. Heat processing enhanced firmness and degree of shrinkage of *Penaeus japonicus* (Mizuta *et al.*, 1999). Denaturation and disintegrating of perimysium and endomysium collagen, together with the denaturation of myofibrils might result in the shrinkage of muscle fibers.

For the transverse sections, gaps between muscle fibers and bundles were visible in raw meats of black tiger shrimp and white shrimp (Figure 15A). Similar microstructure between raw meats of black tiger shrimp and white shrimp were found. Denser structure was noticeable in tail portion of both species. Cooked meats of black tiger shrimp and white shrimp had more compact fiber arrangements, compared with raw samples (Figure 15B). Nip and Moy (1988) reported the microstructural changes of boiled meat prawn (*Macrobrachium rosenbergi*). The more compact fibers might be associated with the increased shear force values of cooked shrimp meats (Figure 11). For the same part tested, no marked differences in microstructure were observed between species. Nevertheless, it seemed to be denser in microstructure for tail portion of both species as manifested by the less gaping of muscle bundle. The differences observed between species and muscle portions might be caused by the difference in muscle fiber size and compositions, particularly the content and nature of intramuscular collagen.

A



B

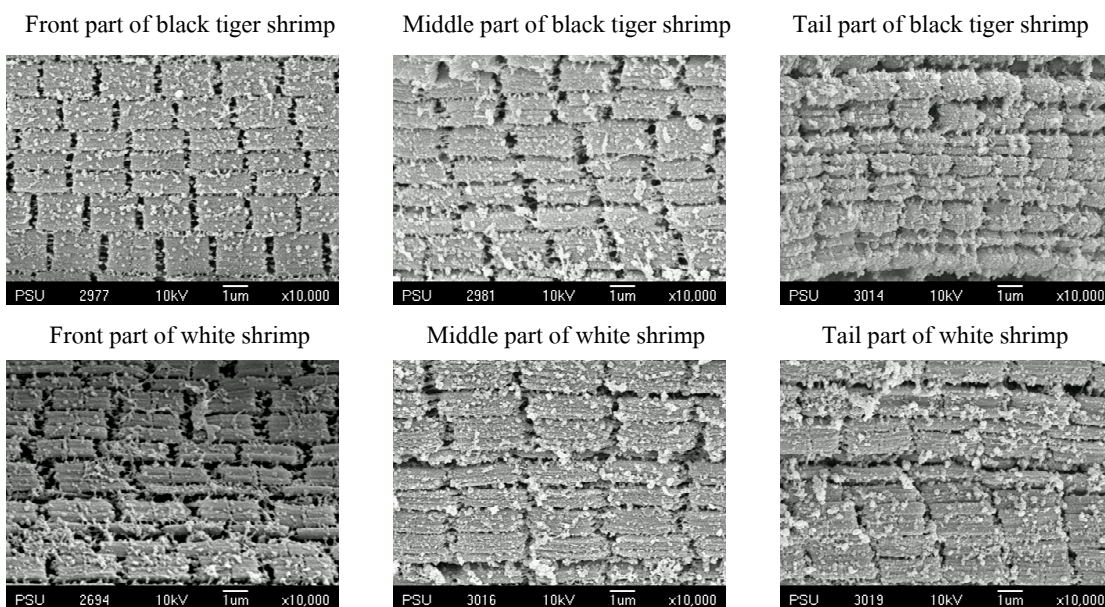
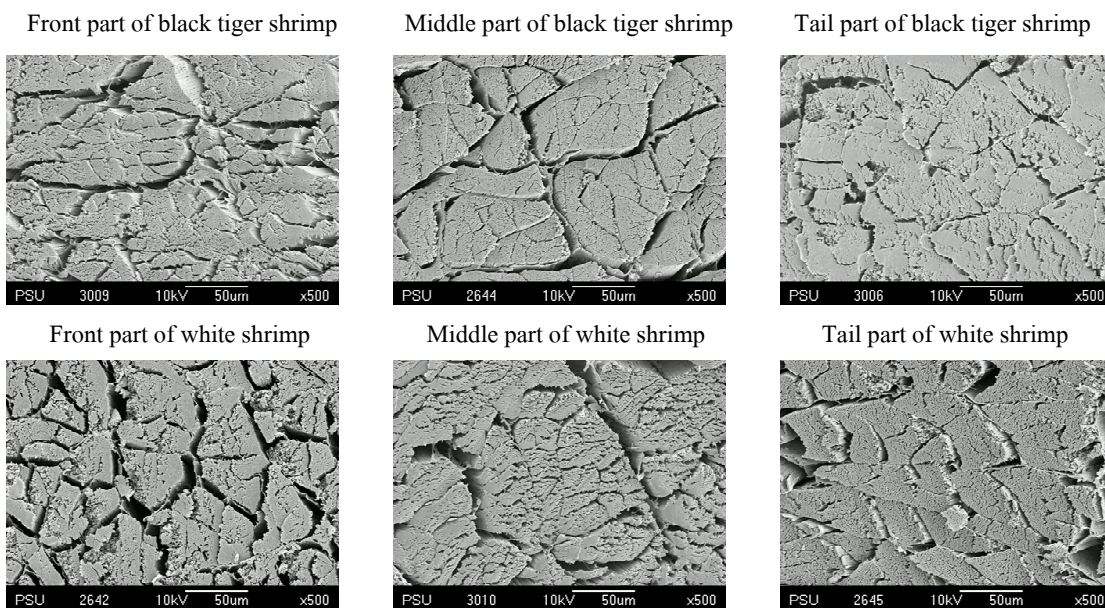


Figure 14 SEM micrographs of longitudinal section of raw (A) and cooked (B) of black tiger shrimp and white shrimp with different parts.

A



B

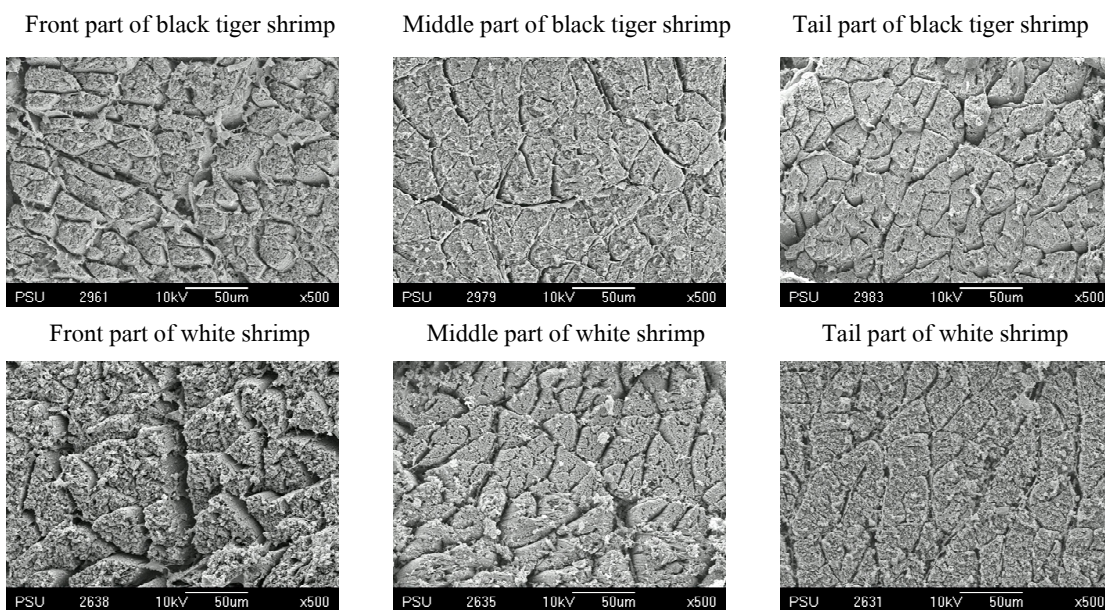


Figure 15 SEM micrographs of transverse section of raw (A) and cooked (B) of black tiger shrimp and white shrimp with different parts.

### 3. Effect of freeze-thawing on physicochemical and physical properties of black tiger shrimp and white shrimp meats

#### 3.1 Effect of freeze-thawing on exudate loss

Exudate losses of black tiger shrimp and white shrimp subjected to multiple freeze-thaw cycles are presented in Table 13. Higher amount of exudate was observed when the freeze-thaw cycles increased ( $p < 0.05$ ). With the freeze-thawing lower than 3 cycles, no differences in exudate were noticeable between both species ( $p > 0.05$ ). Nevertheless, the exudate of white shrimp was greater, compared to that of black tiger shrimp when subjected to 5 cycles of freeze-thawing ( $p < 0.05$ ). The increase in exudate indicates the loss of water holding capacity of muscle. Therefore, the repeated freeze-thawing showed the detrimental effects on the shrimp muscle. Repeated melting and reformation of ice crystals caused the damages of cell membranes and organelles. Freeze-thawing was also shown to increase the cook loss of shrimp muscle (Srinivasan *et al.*, 1997). The drip loss of muscle can lead to less acceptability due to the loss of tasteful constituents, e.g. some amino acids or nucleotides.

Table 13 Exudate loss of black tiger shrimp and white shrimp meats subjected to different freeze-thaw cycles

Freeze-thaw cycles	Shrimp species	
	Black tiger shrimp	White shrimp
0	0.69±0.10aB	0.78±0.02aB
1	0.83±0.04aB	0.87±0.09aB
3	0.92±0.06aB	1.06±0.13aB
5	1.39±0.39bA	2.09±0.37aA

Values are given as mean  $\pm$ SD from triplicate determinations. Different letters in the same row indicate significant differences ( $p < 0.05$ ). Different capital letters in the same column indicate significant differences ( $p < 0.05$ ).

### 3.2 Effect of freeze-thawing on AG and NAG activities

AG and NAG activities in the exudate formed increased when the freeze-thaw cycles increased as shown in Figure 16A and 16B, respectively. At the same freeze-thaw cycle, AG and NAG activities of white shrimp were higher than those found in black tiger shrimp ( $p < 0.05$ ). The differences in activities indicated the differences in stability of shrimp tissues during freeze-thawing process as well as the differences in enzyme activity in the organelles between both species. The formation and acceleration of ice crystals, dehydration and the increase in solute result in the changes in muscle tissues (Shenouda, 1980). The cell damage of muscle was mainly attributed to ice crystal growth as well as the increased salt concentration in the unfrozen phase. Therefore, freezing can disrupt muscle cells, resulting in the release of mitochondrial and lysosomal enzymes into sarcoplasm (Hamm, 1979). AG and NAG have been used as the marker of freezing and thawing process of fish muscle (Benjakul and Bauer, 2000; Rehbein, 1979; Shimomura *et al.*, 1987). From the result, white shrimp muscle tissues were more susceptible to damage induced by freeze-thawing than were the tissues of black tiger shrimp. Thus, the quality of black tiger shrimp might be retained during frozen storage and freeze-thawing to a higher degree, compared to white shrimp.

### 3.3 Effect of freeze-thawing on $\text{Ca}^{2+}$ -ATPase Activity

$\text{Ca}^{2+}$ -ATPase activities of NAM extracted from black tiger shrimp and white shrimp muscles subjected to different freeze-thaw cycles are depicted in Figure 17. Decreases in  $\text{Ca}^{2+}$ -ATPase activity were noticeable after 1 cycle of freeze-thawing ( $p < 0.05$ ). Thereafter, no marked changes were found with increasing freeze-thaw cycles up to 5 cycles ( $p > 0.05$ ). After 5 cycles of freeze-thawing, the activities in black tiger shrimp and white shrimp were decreased by 16.39 and 21.98%, respectively. The decrease in  $\text{Ca}^{2+}$ -ATPase activity was possibly due to the conformational changes of myosin globular head as well as the aggregation of this portion (Okada *et al.*, 1986). Rearrangement of proteins via protein-protein interaction induced by freeze-thawing process might contribute to the loss in ATPase activity (Benjakul and Bauer, 2000). From the result, no differences in  $\text{Ca}^{2+}$ -ATPase activity were found between both species regardless of freeze-thawing cycles. Thus, it was most likely that the stability of muscle proteins, especially myosin, toward freeze-thawing process of both species was similar. The result suggested that

muscle proteins, mainly myosin, underwent denaturation to a greater extent with increasing freeze-thaw cycles.

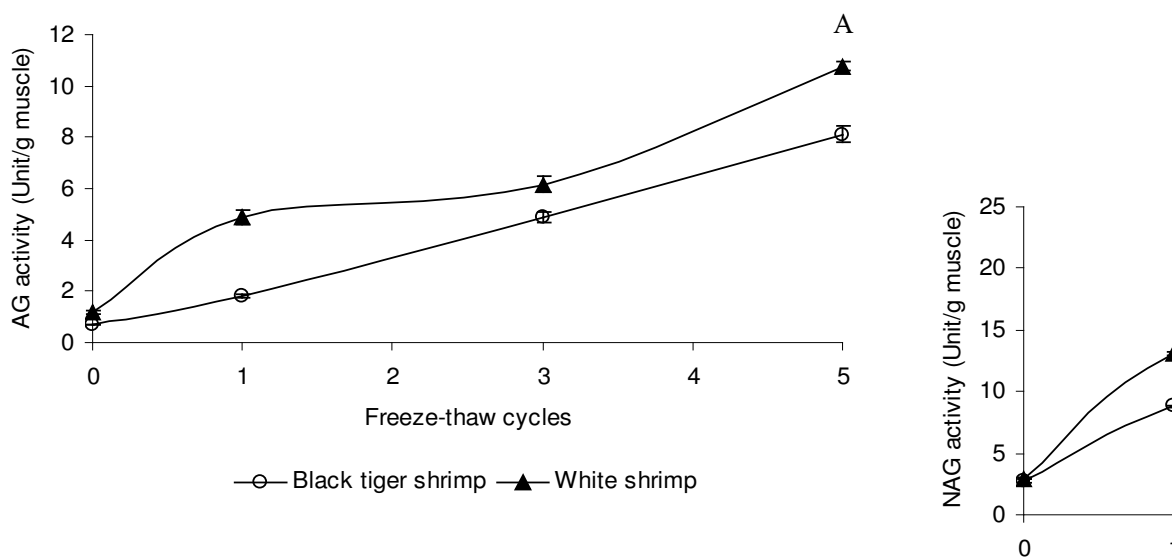


Figure 16 Changes in  $\alpha$ -glucosidase (A) and  $\beta$ -N-acetyl-glucosaminidase (B) activities of black tiger shrimp and white shrimp subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

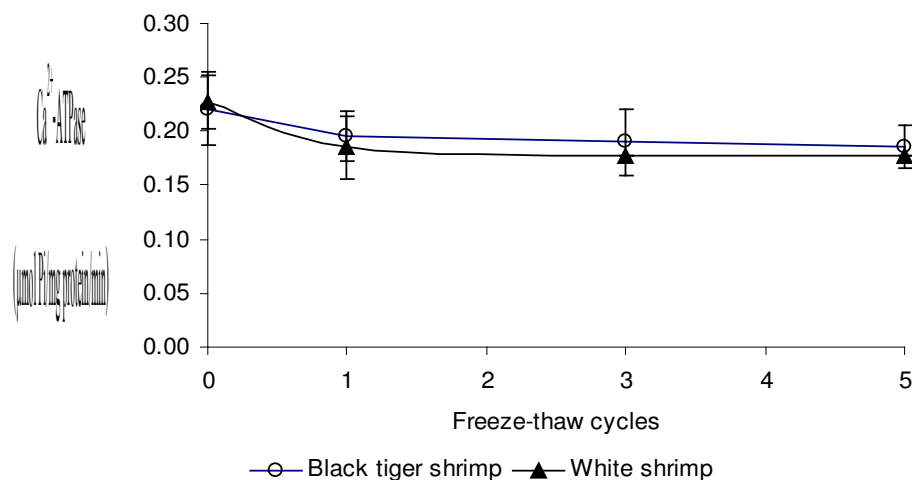


Figure 17 Changes in  $\text{Ca}^{2+}$ -ATPase activity of natural actomyosin extracted from black tiger shrimp and white shrimp subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

### 3.4 Effect of freeze-thawing on sulfhydryl and disulfide bond contents

Sulfhydryl (SH) group content of extracted NAM from both shrimps decreased with increasing freeze-thaw cycles ( $p < 0.05$ ) (Figure 18A). The SH group content of black tiger shrimp and white shrimp NAM decreased to 2.88 and 2.46 mole/ $10^5$  g protein, respectively after 5 freeze-thaw cycles. From the result, the decrease in SH group content was in agreement with the increase in disulfide bond content (Figure 18B). The accelerated denaturation of myosin molecules, especially the conformational changes, in which the reactive sulfhydryl groups were exposed to oxidation, might result in the increased disulfide bond formation. Cysteine is perhaps the most susceptible amino acid residue and it is usually one of the first to be oxidized (Thanonkaew *et al.*, 2006). Another sulfur-containing amino acid, methionine, is also readily oxidized to methionine sulfoxide derivative (Vogt, 1995.) The amino acid with reactive side chain (sulfhydryl, thioether, amino group, imidazole ring and indole ring) are particularly susceptible to oxidation initiated by oxidizing lipid and their products (Gardner, 1979). Oxidized myofibrils showed substantial changes in sulfhydryls and disulfide bonds (Xiong, 2000). From the result, white shrimp tended to have the higher decrease in sulfhydryl group content with the greater disulfide bond content, compared with black tiger shrimp, particularly after 5 cycles of



freeze-thawing. Therefore, white shrimp was possibly more prone to sulfhydryl oxidation than was black tiger shrimp. Benjakul *et al.* (2003) reported that the difference in sulfhydryl content among species during frozen storage was due to the differences in susceptibility in sulfhydryl oxidation of myofibrillar proteins.

### **3.5 Effect of freeze-thawing on surface hydrophobicity**

The changes in surface hydrophobicity ( $S_0$ ANS) in extracted NAM from both shrimps as influenced by freeze-thaw cycles are shown in Figure 19. In general, surface hydrophobicity of NAM from black tiger shrimp and white shrimp increased when the freeze-thaw cycles were greater than 3 cycles. After 5 cycles, surface hydrophobicity of NAM from both species increased by 21.91% and 37.90%, respectively. The polar (hydrophilic) residues are generally exposed to water, while the nonpolar (hydrophobic) groups or moieties are generally localized in the molecule. An increase in  $S_0$ ANS, regardless of the cause, is assumed to result from structural alterations, which, in some cases, would mean an irreversible denaturation (Nakai and Li-Chan, 1988). After 5 cycles of freeze-thawing,  $S_0$ ANS of white shrimp was greater than that of black tiger shrimp, suggesting that the former was more prone to the conformational changes induced by repeated freeze-thawing than was the latter.

### **3.6 Effect of freeze-thawing on solubility**

Protein solubility in 0.6 M KCl of black tiger shrimp and white shrimp muscle subjected to multiple freeze-thaw cycles is depicted in Figure 20. Protein solubility of both shrimps slightly decreased when the freeze-thaw cycles increased ( $p < 0.05$ ). White shrimp had the greater decrease in protein solubility than did black tiger shrimp, particularly after 5 freeze-thaw cycles ( $p < 0.05$ ). The decrease in solubility was in accordance with the increase in surface hydrophobicity (Figure 19). The loss in salt soluble protein suggested that protein denaturation was induced by the freeze-thawing process. The decrease in solubility was most likely associated with the formation of disulfide bond (Figure 18). The decrease in solubility of protein has been used as a marker of oxidative deterioration of muscle protein (Decker *et al.*, 1993; Srinivasan and Hultin, 1997; Xiong and Decker, 1995). Thermodynamically, a decrease in protein solubility is the result of a shift from a balance of protein intermolecular interaction and protein–water interaction, resulting in a situation, where protein intermolecular interaction is forced, while protein water interaction is weakened (Vojdani, 1996). As a result of loss of ordered tertiary

structure, the cross-linkages are formed among proteins as evidenced by the decrease in solubility. Free radical attack is also a major cause of decreased protein solubility (Decker *et al.*, 1993). The decrease in solubility was also associated with the increase in exudate of both species with increasing freeze-thaw cycles (Table 13)

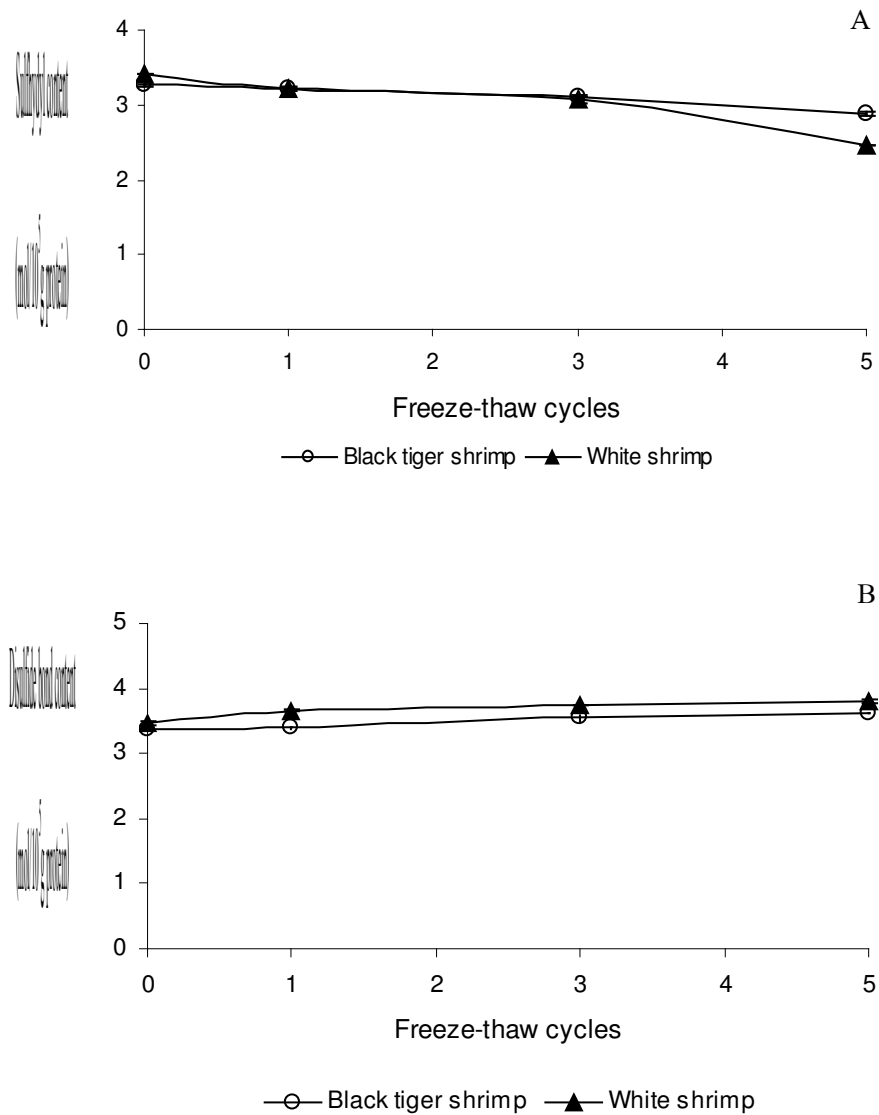


Figure 18 Changes in sulfhydryl group content (A) and disulfide bond content (B) of natural actomyosin extracted from black tiger shrimp and white shrimp subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

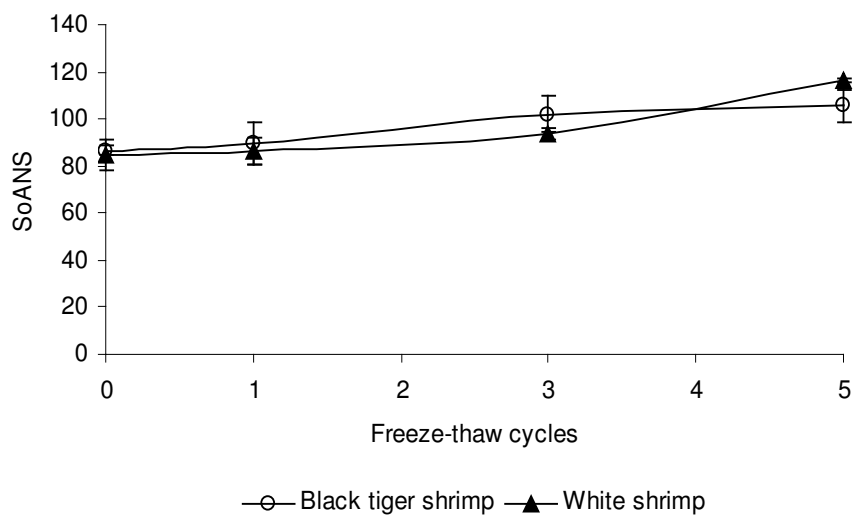


Figure 19 Changes in surface hydrophobicity of natural actomyosin extracted from black tiger shrimp and white shrimp subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

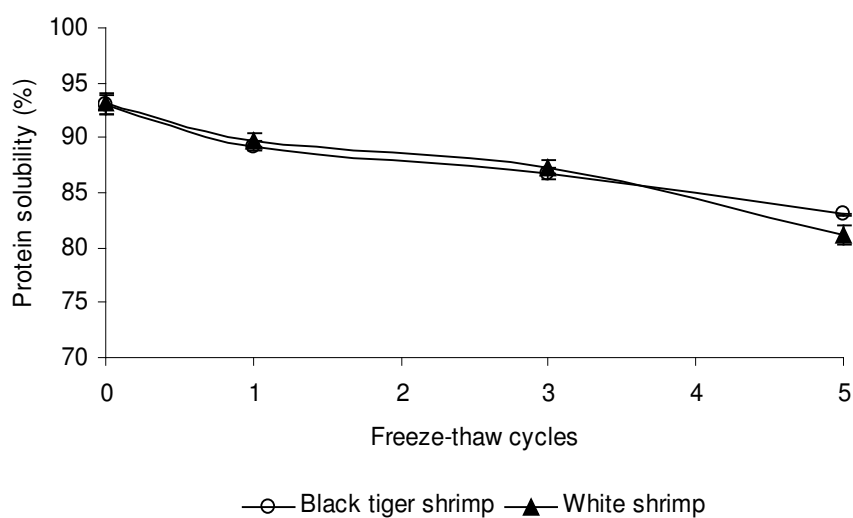


Figure 20 Changes in protein solubility of black tiger shrimp and white shrimp subjected to different freeze-thaw cycles. Bars represent the standard deviation from triplicate determinations.

### 3.7 Effect of freeze-thawing on shear force

Shear force of black tiger shrimp and white shrimp before and after subjecting to 5 freeze-thaw cycles is presented in Figure 21. Decreases in shear force of both shrimps were found after freeze-thawing ( $p < 0.05$ ). The shear force value of white shrimp was lower, compared to that of black tiger shrimp regardless of freeze-thawing ( $p < 0.05$ ). The decrease in shear force suggested the loss in integrity of muscle fibers, leading to the weakening of muscle. Repeated melting and reformation of ice crystals caused the damages of cell membranes, organelles as well as muscle structure. Shear force correlated either with the diameter (width) of the muscle portion sheared or with the weight of the shrimp (Srinivasan *et al.*, 1997). Size of muscle or bundles had a major effect on shrimp meat tenderness (Srinivasan *et al.*, 1997). Due to the same size of shrimp used, the differences in shear force might be caused by the different composition, particularly collagen content. Differences in microstructure, particularly the arrangement of muscle fibers between both species were also postulated. Therefore, freeze-thawing showed the detrimental effect on shrimp texture as manifested by the lowered shear force.

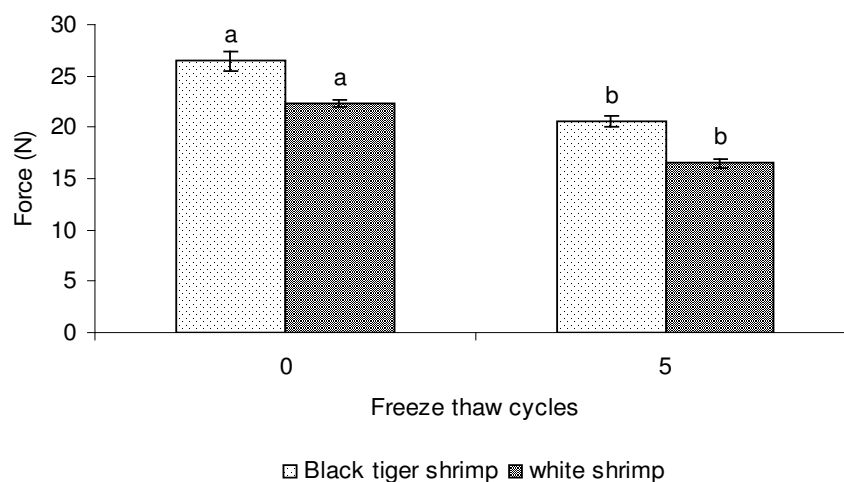


Figure 21 Changes in shear force of black tiger shrimp and white shrimp subjected to different freeze-thaw cycles. The same letters within the same treatment indicate non significant differences ( $p > 0.05$ ). Bars represent the standard deviation from triplicate determinations.

### 3.8 Effect of freeze-thawing on microstructure

Microstructures of black tiger shrimp and white shrimp subjected to 5 freeze-thaw cycles in comparison with fresh shrimp are shown in Figure 22A and 22B. For the longitudinal sections, shrinkage of fibers was noticeable as evidenced by the gaping formed (Figure 16A). Also the losses of Z-disks were also observed after freeze-thawing. However, the destruction of Z-disks was more pronounced in white shrimp after freeze-thawing. It was reported that freeze-thawing process caused the shrinkage and drip loss of muscle fibers (Hale and Waters, 1981). Cross-linking of myosin heavy chain through disulfide and nondisulfide covalent bonds during frozen storage contributed to the formation of high-molecular-weight polymers and aggregates (Ragnarsson and Regenstein, 1989). This might be associated with the shrinkage of muscle fibers. For the transverse section, muscle bundles were more separated when subjected to 5 freeze-thaw cycles (Figure 16B). Protein denaturation and disruption of endomysium induced by freeze-thawing possibly resulted in the less compact structure. The looser structure and disruption of muscle fibers was coincidental with the lower shear force value of both shrimps. The disrupted structure together with the denaturation of myofibrillar protein induced by freeze-thawing were most likely associated with the lowered water holding capacity of muscle as shown by the higher drip loss (Table 13).

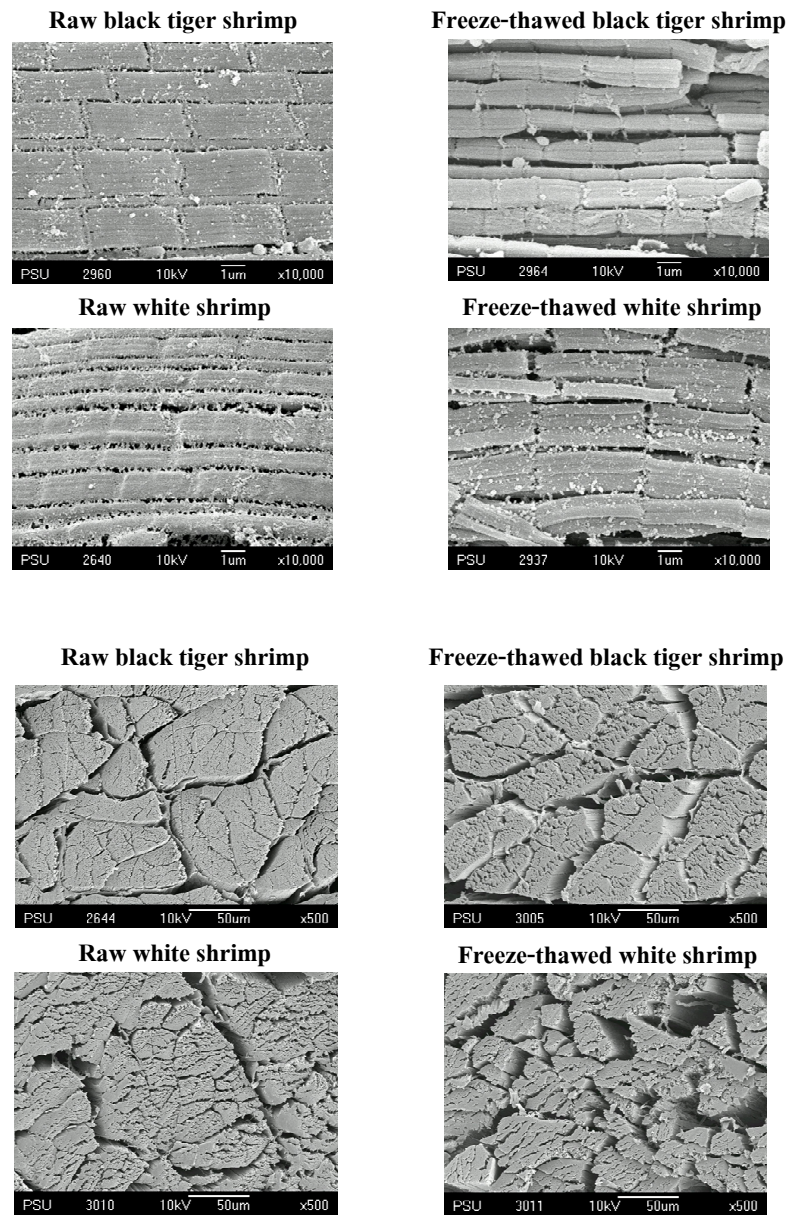


Figure 22 Microstructure of black tiger shrimp and white shrimp before and after subjecting to 5 freeze-thaw cycles; longitudinal section (A) and transverse section (B).

#### **4. Effect of ice storage on quality changes of black tiger shrimp and white shrimp**

##### **4.1 Chemical changes of black tiger shrimp and white shrimp during iced storage**

###### **4.1.1 Changes in pH**

A gradual increase in the pH was observed in black tiger shrimp and white shrimp meat up to 8 days of iced storage ( $p < 0.05$ ) (Figure 23A and 23B). Generally, the pH of black tiger shrimp increased sharply within the first two days and remained constant after 8 days. For white shrimp, pH increased gradually up to 10 days of storage and no change was found thereafter ( $p > 0.05$ ). The differences in pH changes between both species might be due to the differences in buffering capacity of muscle, which was presumably greater in white shrimp. The increase in pH was postulated to be due to an increase in volatile bases produced by either endogenous or microbial enzymes. Benjakul *et al.* (2002) reported that the decomposition of nitrogenous compounds caused an increase in pH in fish flesh. The increase in pH ranging from 0.1 to 0.2 units represented a first quality muscle with the acceptable quality and the increase higher than 0.2 units indicated the deteriorated samples (Riaz and Quadri, 1985). Rahaman *et al.* (2001) reported that the pH value of shrimp (*Penaeus monodon*) varied from 6.63 to 7.95 during 10 days of iced storage. Changes in pH might be different depending on a variety of factors such as species, fishing ground, feeding, storage temperature and buffering capacity of meat (Pacheco-Aguilar *et al.*, 2000). Changes in pH also depended on the liberation of inorganic phosphate and ammonia due to the enzymatic degradation of ATP (Sikorski *et al.*, 1990). From the result, the initial pH between two species was similar. However, black tiger shrimp muscle had a higher increase in pH than did white shrimp muscle during storage times. Apart from the different buffering capacity, the activity of enzymes converting glycogen into lactic acid might be different between two species. Lactic acid, generated in anoxic conditions from glycogen, is the principal factor in lowering the post mortem pH in the fish muscles (Sikorski *et al.*, 1990). From the result, no marked differences in pH were found among all treatments of the same species throughout the storage ( $p > 0.05$ ), except that whole white shrimp had the higher pH than did headless samples, regardless of icing method.

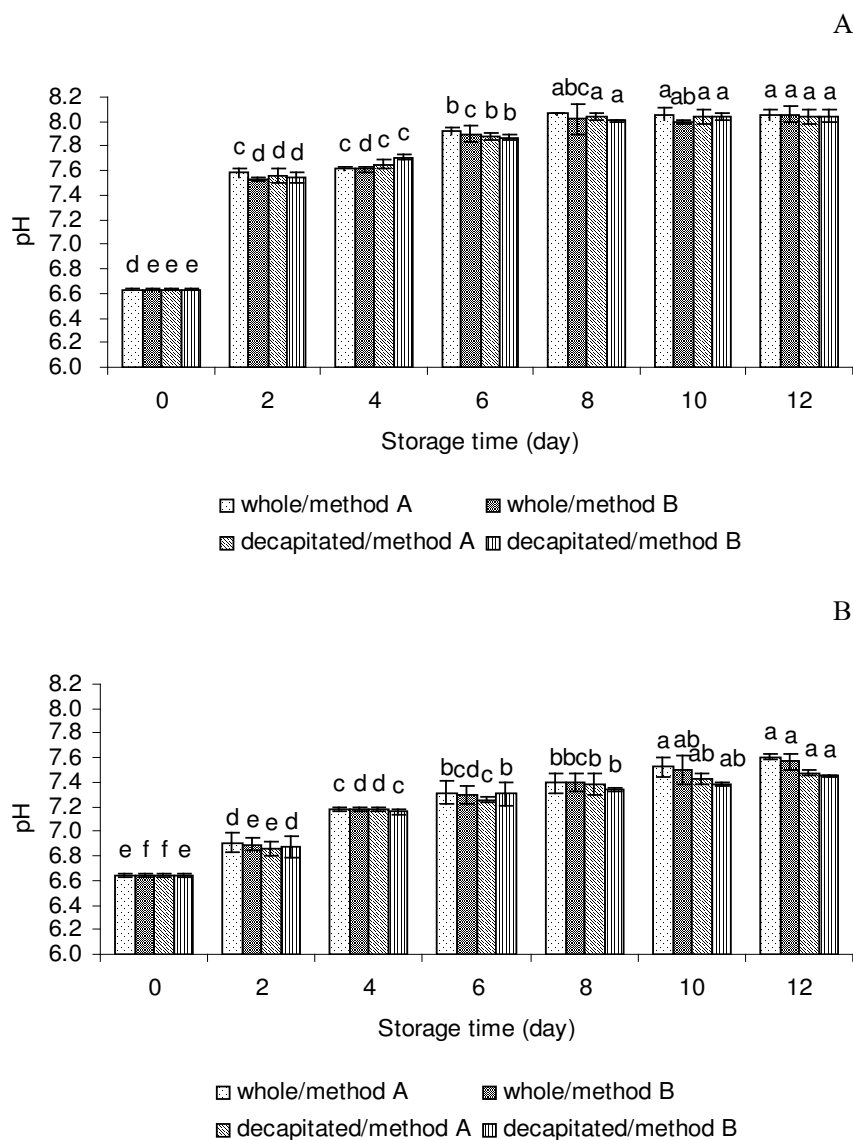


Figure 23 Changes in pH of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the same treatment indicate non significant differences ( $p > 0.05$ ). Bars represent the standard deviation from triplicate determinations.

#### 4.1.2 Changes in TVB-N and TMA-N

Changes in TVB-N of black tiger shrimp and white shrimp during iced storage are shown in Figure 24A and 24B, respectively. TVB-N content of decapitated shrimps of both species was generally lower than those of whole shrimps during 12 days of storage in ice ( $p < 0.05$ ). The initial values of TVB-N of black tiger shrimp and white shrimp were 3.30 and 2.59



mg N/100g sample, respectively. The initial TVB-N of black tiger shrimp storage in ice was 5.88 mg N/100g sample (Rahman *et al.*, 2001). The differences in TVB-N might be due to the varying handling process or storage prior to analysis. From the result, TVB-N of shrimp samples varied with treatments. The increases in TVB-N of whole samples occurred at a faster rate than those of decapitated shrimps regardless of icing method ( $p < 0.05$ ). Owing to the fact that digestive organs, which are the major sources of enzymes, are localized in the cephalothorax portion, the removal of cephalothorax presumably resulted in less hydrolysis of nitrogenous compounds. As a consequence, the microbial load could be lowered due to the lower content of low MW compounds, normally used for microbial growth. Among all samples, the highest TVB-N value was found in whole black tiger shrimp with icing method A. During extended storage in ice, the autolysis and microbial spoilage were more pronounced as evidenced by the increase in TVB-N contents. Based on TVB-N, both shrimps could be justified as acceptable for consumer since TVB-N content was lower than 10 mg/100g in all samples stored in ice up to 12 days. The recommended TVB-N value of 30 mg/100g was reported previously by Connell (1995).

TMA-N of black tiger shrimp and white shrimp increased sharply within the first 2 days of storage as shown in Figure 25A and 25B, respectively. Production of TMA-N in muscle during cold storage could be used as an indicator of bacterial activity (Gokodlu *et al.*, 1998). Subsequently, a slight increase in TMA-N was observed in black tiger shrimp up to 12 days of storage. However, no changes in TMA-N were noticeable in white shrimp during 2 to 12 days of storage ( $p > 0.05$ ). TMAO-N is broken down to TMA by psychotropic bacterial enzymes during ice storage (Yamagata and Low, 1995). The result suggested that the spoilage of shrimps, particularly black tiger shrimp, caused by bacteria with TMAO reducing activity occurred when the storage time increased. Additionally, the formation of TVB and TMA is generally associated with the growth of specific spoilage bacteria such as *Shewanella putrefaciens*, *Photobacterium phosphoreum*, and *Vibrionaceae* (Gram and Huss, 1996). The lower TMA content in both species might result from the lower TMAO. From the result, the formation of TMA was coincidental with the occurrence of TVB, especially when the storage time increased. The increases in both TVB and TMA were also in accordance with the increases in pH (Figure 23).

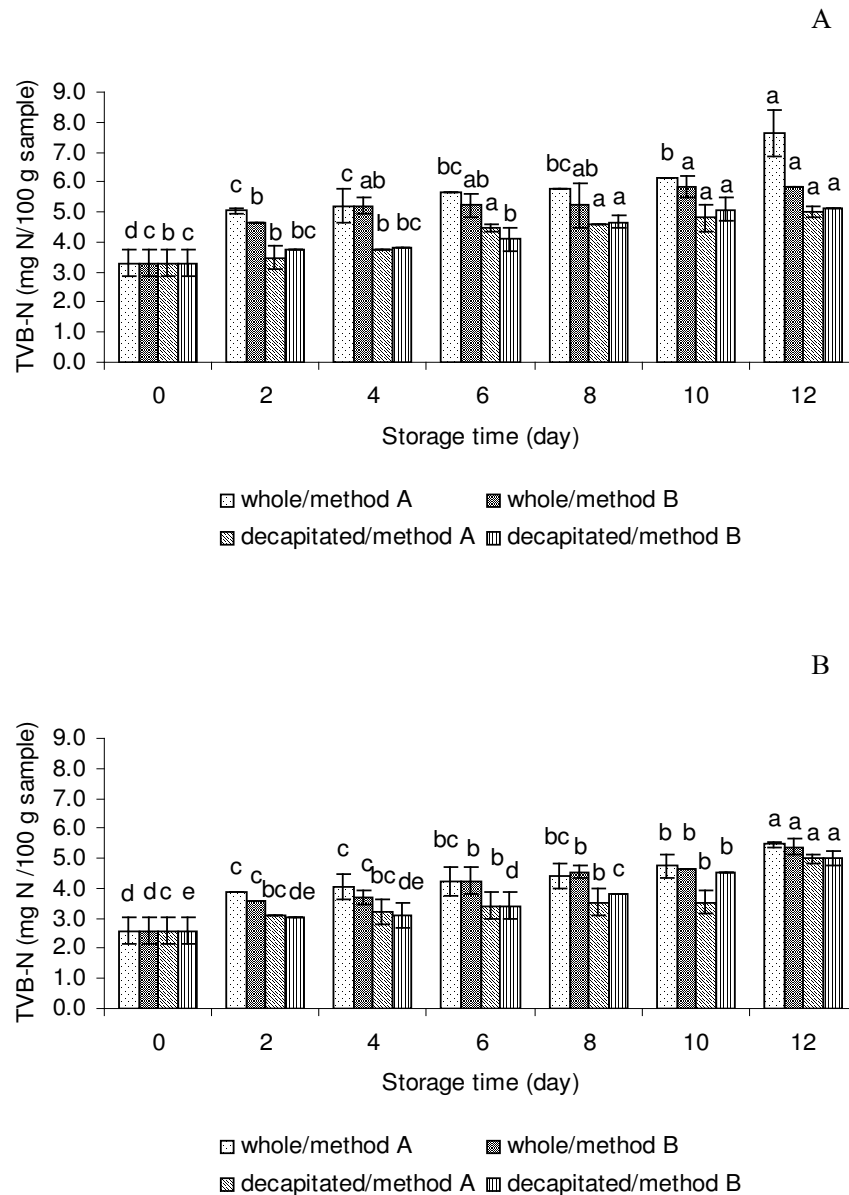


Figure 24 Changes in TVB-N of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the same treatment indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.

From the result, whole black tiger shrimp stored with icing method A tended to contain higher TMA. Molten ice could be a source of microorganism having TMAO reductase. As a result, TMAO was more reduced to TMA. Therefore, the drainage should pave a way to

extend the shelf-life of whole shrimp. When comparing TVB and TMA contents between both species under the same pretreatment condition, black tiger shrimp muscle showed the higher contents of TVB and TMA during iced storage ( $p < 0.05$ ). From the results, pretreatment of shrimp by decapitation could retard the spoilage of shrimp during iced storage. Limits of TVB-N of 30 mg N/100g and TMA-N of 5 mg N/100g in shrimp have been reported (Cobb *et al.*, 1973).

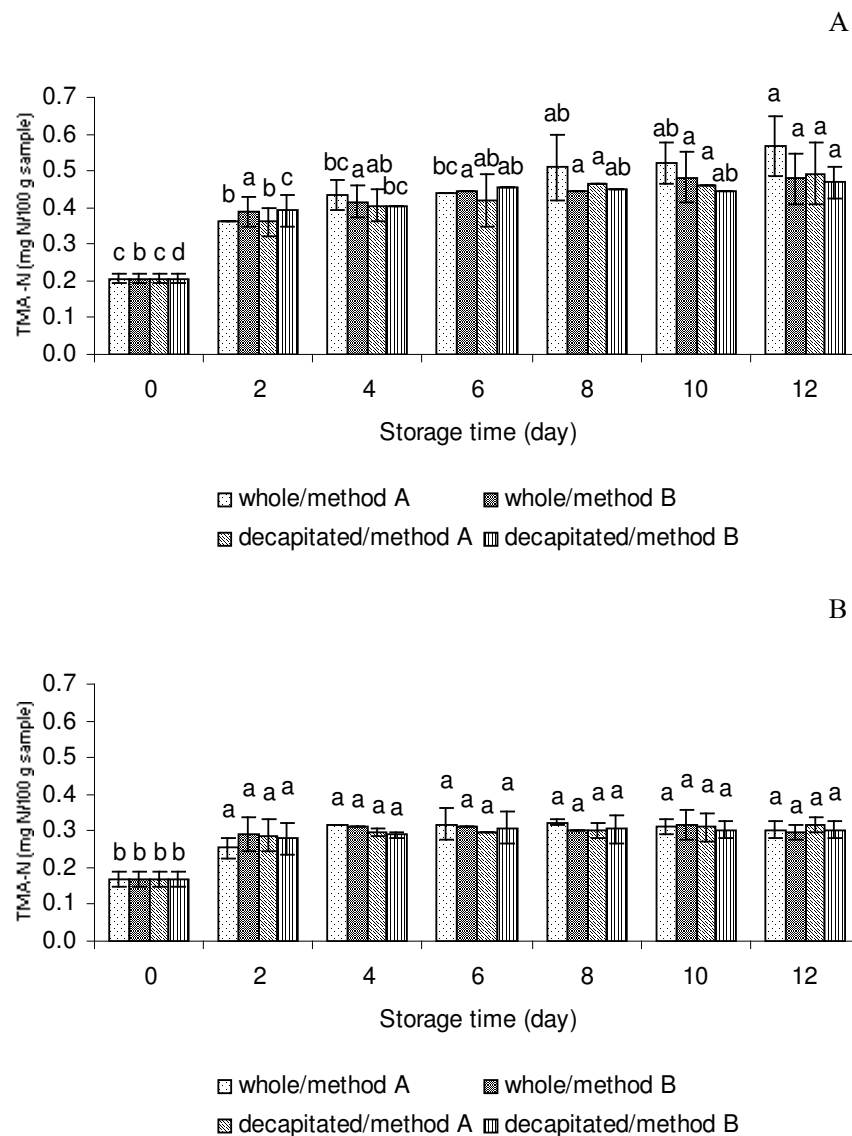


Figure 25 Changes in TMA-N of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the same treatment indicate non significant differences ( $p > 0.05$ ). Bars represent the standard deviation from triplicate determinations.

#### 4.1.3 Changes in biogenic amines

Changes in biogenic amines of black tiger shrimp and white shrimp during iced storage are shown in Table 14 and 15, respectively. Only spermine was observed in fresh black tiger shrimp at day 0 (Table 14), but it was not detectable in fresh white shrimp (Table 15). Spermidine and cadaverine were found in all black tiger shrimp samples at day 4, except whole black tiger shrimp with icing method B and the decapitate sample with icing method A. All black tiger shrimps had putrescine ranging from 1.80 to 2.26 ppm at day 8 of storage. No tyramine was found in black tiger shrimp. At day 12, whole black tiger shrimp with icing method A contained the extremely high putrescine (129.37 mg/kg). Generally, icing method B could retard the formation of biogenic amines in black tiger shrimp effectively. Bacteria belonging to the family Enterobacteriaceae have ability to produce both cadaverine and putrescine (Moori *et al.*, 1988). For white shrimp, whole shrimp stored in ice with method A contained putrescine, cadaverine, histamine and spermidine at day 4 of storage. However, no putrescine, cadaverine, histamine and tyramine were found in other samples at the same period of storage. With decapitation, the formation of biogenic amines was more retarded. From the result, decapitated white shrimp stored in ice with method B was shown to have the lowest biogenic amines quantitatively and qualitatively. Among the amines, histamine has been more frequently implicated in food poisoning, and the diamines, primarily putrescine and cadaverine, are known to enhance histamine poisoning (Bjeldanes *et al.*, 1978). Earlier Moori *et al.* (1988) pointed out that, at low temperature, the bacteria belonging to the family Enterobacteriaceae are not very active in forming the amines. Due to the presence of putrescine in all samples, particularly with increasing storage time, putrescine-forming bacteria could grow at this storage temperature and contribute to the formation of putrescine. In shrimp, putrescine has been suggested as an index of decomposition (Shakila *et al.*, 1995). Putrescine at level of 3 ppm was regarded as the rejection point of unprocessed shrimp (Penaeid Shrimp) (Benner *et al.*, 2003). Histamine and tyramine were found in white shrimp, but were not detected in black tiger shrimp during storage. The absence of histamine-forming bacteria in shrimp was presumed and a low level of histidine in shrimp muscle was also postulated. Middlebrooks *et al.* (1988) reported that in fish decomposed at 0°C, the levels of cadaverine and putrescine began to rise sharply at about 11 days of incubation, while histamine appeared only after 16 days. Amines are formed during spoilage of

fish as a result of bacterial decarboxylation of free amino acids (Lakshmanan *et al.*, 2002). Putrescine, cadaverine, histamine, agmatine, tyramine, tryptamine and 2-phenylethylamine are the decarboxylation product of ornithine, lysine, histidine, arginine, tyrosine, tryptophan and phenylalanine, respectively (Ozogul *et al.*, 2006).

Quality index (QI) of black tiger shrimp stored in ice increased slightly within the first 8 days. The sharp increase in QI was noticeable in sample kept in ice with method A, particularly for whole shrimp sample. The similar result was observed for biogenic amine index (BAI). Mietz and Karmas (1997) proposed the biogenic amines index (BAI) to evaluate the quality of rockfish, salmon, lobster and shrimp. The highest BAI of both shrimps were found at day 12 except the decapitated shrimp stored in ice with method A. The increases in QI and BAI indicated the spoilage of black tiger shrimp. For white shrimp, the increases in both QI and BAI were found in samples stored in ice for a longer time. However, the highest QI and BAI in white shrimp were found in the decapitated sample stored in ice with method A. For white shrimp, the decapitation might cause the contamination of microorganisms particularly from the retained molten ice. Thus, the growth of biogenic amine formers might be enhanced. As a result, the greater formation of biogenic amine was observed in this sample. However, QI of all treatments of both species was not greater than 10. Mielitz and Karmas (1977) proposed the value of 10 as the limit of fish acceptability for QI. Thus, those shrimp were still acceptable after keeping in ice for up to 12 days.

Table 14 Changes in biogenic amine contents (mg/kg) of whole and decapitated black tiger shrimp with different icing methods during the storage

Treatment	Day	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	QI	BAI	
Whole/method A	0	-	-	-	-	-	11.54±0.26	0.00	0.00	
	4	-	1.82±0.00	-	-	0.78±0.24	7.19±0.03	0.20	1.82	
	8	2.23±0.39	2.45±0.17	-	-	-	14.40±0.12	0.30	4.68	
	12	129.37±2.64	2.13±0.17	-	-	1.09±0.28	11.88±4.93	9.41	131.50	
	Whole/method B	0	-	-	-	-	-	11.54±0.26	0.00	0.00
Whole/method B	4	-	-	-	-	2.25±0.00	12.79±0.26	0.00	0.00	
	8	2.15±0.62	1.39±0.07	-	-	1.92±0.00	6.70±2.19	0.37	3.54	
	12	3.56±0.55	-	-	-	1.77±0.22	11.05±4.10	0.26	3.56	
	Decapitated/method A	0	-	-	-	-	-	11.54±0.26	0.00	0.00
		4	-	-	-	-	1.75±0.00	10.51±0.26	0.00	0.00
8		2.26±0.00	2.22±0.00	-	-	1.25±0.17	11.58±0.71	0.32	4.48	
12		13.94±0.01	2.59±0.21	-	-	1.04±0.00	10.99±0.14	1.27	16.53	
Decapitated/method B		0	-	-	-	-	-	11.54±0.26	0.00	0.00
	4	-	3.16±0.08	-	-	1.71±0.10	15.59±1.00	0.17	3.16	
	8	1.80±0.04	2.08±0.05	-	-	1.47±0.00	8.63±6.82	0.35	3.88	
	12	3.72±0.16	-	-	-	1.48±0.11	13.03±0.27	0.24	3.72	

Values are given as mean ±SD from duplicate determinations.

#### 4.1.4 Changes in glycogen

Changes in glycogen content of black tiger shrimp and white shrimp meats during iced storage are shown in Figure 26A and 26B, respectively. The decreases in glycogen of black tiger shrimp and white shrimp meat were found up to 6 days of storage ( $p < 0.05$ ). The differences in glycogen content between treatments were observed during 2 – 4 days of storage. At day 2, whole black tiger shrimp showed the lower glycogen content than did the decapitated samples ( $p < 0.05$ ). At day 4, whole white shrimps contained the lower glycogen content than did the decapitated sample ( $p < 0.05$ ). At day 0, it was noted that glycogen content between both species was different. Black tiger shrimp comprised the higher glycogen content (6.96 mg/100g dw) than did white shrimp (5.20 mg/100g dw). The differences might be due to the differences in

feeding behavior, struggling before death, glycolysis, etc. Glycogen of both shrimps possibly underwent glycolysis after death, resulting in the lower remaining glycogen. Baden *et al.* (1994) reported that glycogen in Norway lobster was more readily accessible when there was a shift to anaerobic metabolism. Glycogen concentrations in muscle of shrimp were very low (Cuzon and Aquacop, 1998). Dall (1965) reported that glycogen was not particularly abundant in shrimp (*Metapenaeus* sp.).

Table 15 Changes in biogenic amine contents (mg/kg) of whole and decapitated white shrimp with different icing methods during the storage

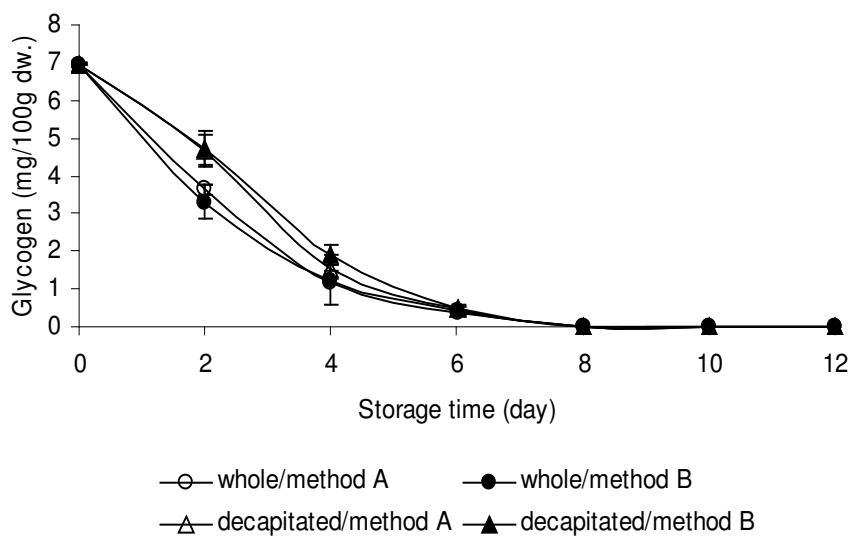
Treatment	Day	Putrescine	Cadaverine	Histamine	Tyramine	Spermidine	Spermine	QI	BAI
Whole/method A	0	-	-	-	-	-	-	0	0
	4	0.62±0.28	1.30±0.64	0.72±0.12	-	2.21±1.26	4.17±1.00	0.08	0.62
	8	0.88±0.24	-	-	-	1.75±0.08	8.65±0.92	0.08	0.88
	12	27.85±0.18	1.52±0.02	-	1.33±1.88	1.02±0.10	5.93±0.33	3.70	30.69
Whole/method B	0	-	-	-	-	-	-	0	0
	4	-	-	-	-	1.50±0.23	4.49±0.60	0	0
	8	1.31±0.14	-	-	-	1.37±0.33	7.28±2.35	0.14	1.31
	12	3.84±0.39	1.15±1.63	0.66±0.93	-	1.87±0.69	7.33±3.35	0.55	5.66
Decapitated/method									
A	0	-	-	-	-	-	-	0	0
	4	-	-	-	-	1.29±0.00	2.66±1.07	0	0
	8	1.68±0.20	-	-	-	1.19±0.08	8.22±1.43	0.16	1.68
	12	60.62±2.30	1.55±0.20	-	0.61±0.86	2.03±0.04	7.42±1.31	5.95	62.78
Decapitated/method									
B	0	-	-	-	-	-	-	0	0
	4	-	-	-	-	1.10±0.06	2.02±0.77	0	0
	8	1.42±0.14	-	-	-	1.46±0.05	14.10±1.35	0.09	1.42
	12	5.72±0.00	-	-	-	1.01±0.00	6.08±0.00	0.71	5.72

Values are given as mean ±SD from duplicate determinations.

From the result, decomposition of glycogen was noticeable during 6 days of storage and no glycogen was found after 6 days. The degradation of glycogen during storage led

to the accumulation of lactate (Gruschczyk and Kamp, 1989). When comparing the glycogen content of samples with different treatments, it was found that the decapitated sample tended to contain the higher amount of glycogen retained during iced storage. Enzyme associated with glycolysis found in cephalothorax could be removed with decapitation process. As a result, higher glycogen content was observed in the decapitated samples.

A



B

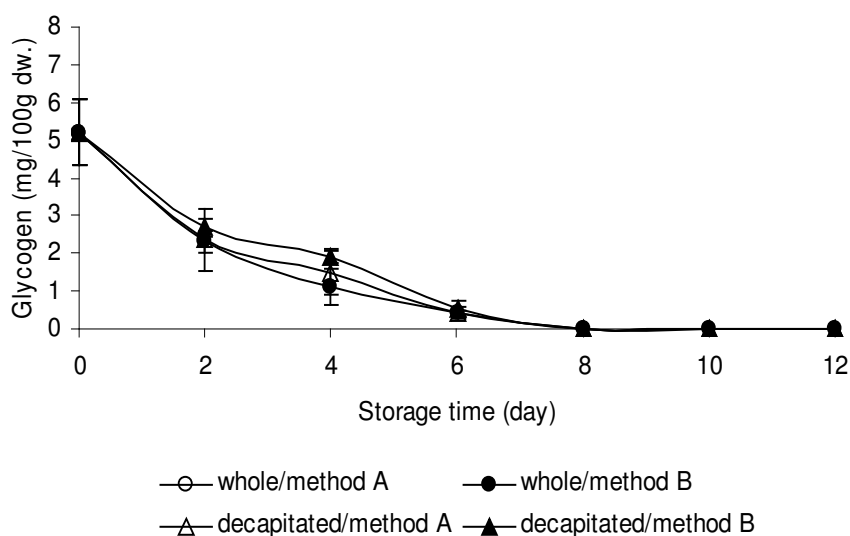


Figure 26 Changes in glycogen of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations.



#### 4.1.5 Changes in K-value

K-value, which is a ratio of inosine (Ino) and Hypoxanthine (Hyp) to the sum of ATP, ADP, AMP, IMP, Ino and Hyp, has been proposed as freshness index of seafood (Saito *et al.*, 1959). As both shrimps were kept in ice for a longer time, K-value increased continuously ( $p < 0.05$ ). At day 0, K-value of black tiger shrimp and white shrimp was 0.29 and 0.70, respectively. Low K-value of both shrimps confirmed that samples used in the study were very fresh. Only slight increases in K-value of both species were found at day 2 of storage. Lowering the temperature reduces enzyme activities and consequently has a major impact on the patterns of purine compounds in stored shrimp (Lou, 1998). Sharp increases in K-value of both shrimps were observed after 4 days of storage time ( $p < 0.05$ ). From the result, whole black tiger shrimp and white shrimp kept in ice with method A had K-value of more than 10% at day 4 of storage. The initial value of K in fish muscle immediately after capture was reported not to exceed 10% (Sikorsky *et al.*, 1990). During the extended storage, the continuous increases in K-value of both shrimps were observed ( $p < 0.05$ ). The degradation of AMP to IMP proceeded by endogenous enzymes in the kuruma prawn muscle (Matsumoto and Yamanaka, 1991). The higher K-value of whole sample of both species was generally observed in comparison with the decapitated samples. The increase in K-value was more pronounced in samples stored in ice with method A. During storage, the ice was molten and retained in the container. As a consequence, the microorganisms, particularly spoilage bacteria, might be accumulated. Those microorganisms possibly produced the glycolytic enzymes, which could lower glycogen content. However, no marked differences in K-value were found between species during storage, except that white shrimp showed the higher K-value than did black tiger shrimp at day 8 of storage. Based on K-value, both shrimps could be justified as acceptable for consumer since K-value lower than 60% was found in all samples stored in ice up to 12 days, regardless of decapitation and icing methods. K-value of 20% was regarded as the freshness limit, while 60% was considered as the rejection point (Ehira, 1976).

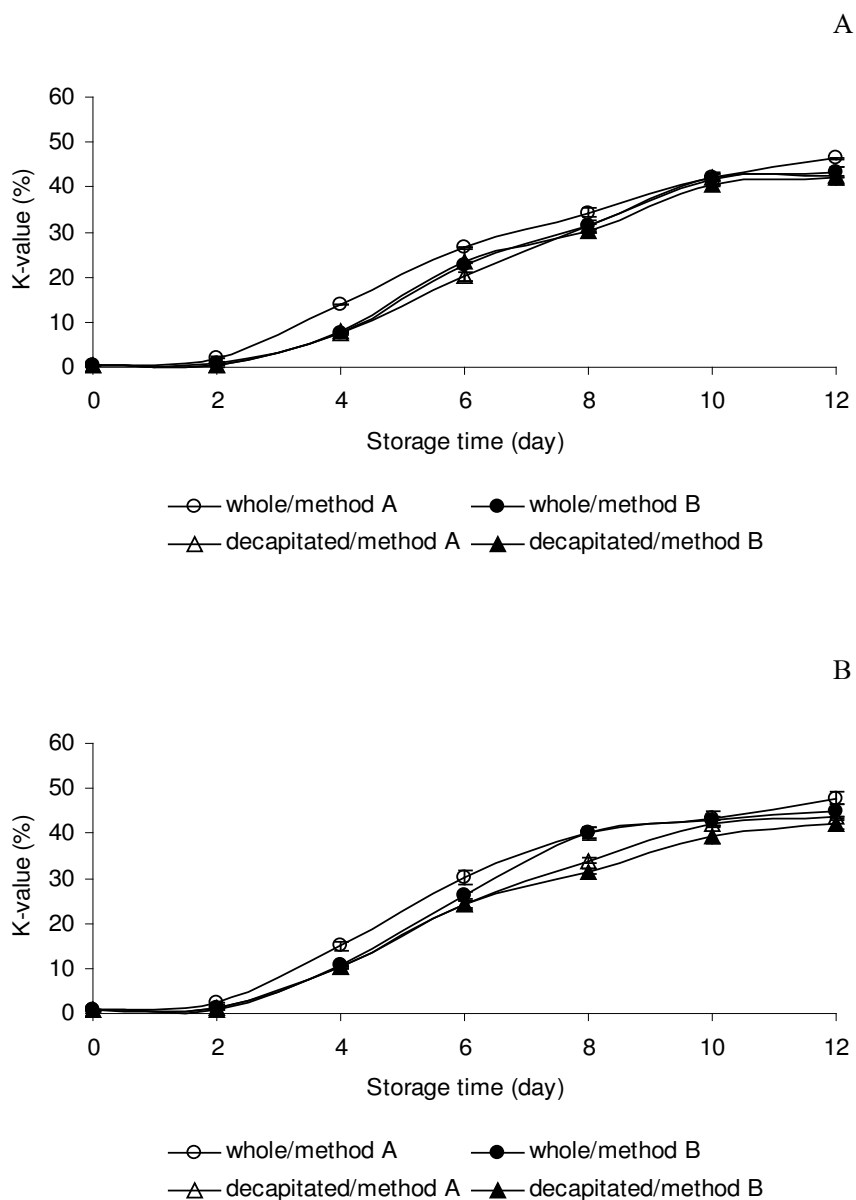


Figure 27 Changes in K-value of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations.

#### 4.1.6 Changes in TBARS

Changes in TBARS of both shrimps during iced storage are depicted in Figure 28. TBARS value in shrimp meat increased as the storage time increased ( $p > 0.05$ ). The initial values of TBARS of black tiger shrimp and white shrimp were 1.40 and 1.16 mg/kg meat,

respectively. It was suggested that lipid oxidation occurred during post-mortem handling to some extent. TBARS index is the most used indicator for advanced lipid oxidation (Nishimoto *et al.*, 1985). TBARS has been used to measure the concentration of relatively polar secondary reaction products, especially aldehydes (Nawar, 1996). The increase in TBARS indicated the formation of secondary lipid oxidation products (Kolakowska, 2002). For black tiger shrimp, no differences in TBARS were observed among all treatments after 4 days of iced storage. However, whole white shrimp stored in ice with method B tended to have higher TBARS, compared with other treatments, particularly during the first 6 days of storage. At 12 days of iced storage, TBARS value in black tiger shrimp and white shrimp muscle increased approximately by 45.72-63.88%, when compared with that found in fresh meat. From the result, it was suggested that lipid oxidation took place in both shrimps during iced storage. The differences in TBARS between both species might be associated with the differences in lipid compositions especially unsaturated fatty acids (Table 9). Due to a high content of phospholipids (Table 8), possibly from the skin and subdermal fat layer, the oxidation of unsaturated fatty acid in phospholipids could take place rapidly (Ke *et al.*, 1977). From the result, lipid oxidation could be retarded to some extent, when the cephalothorax was removed. Lipase and lipoxygenase in the cephalothorax, causing the hydrolysis and oxidation, were postulated to be removed by decapitation. However, both shrimps had TBARS value not more than 3.0 mg malonaldehyde/kg sample during iced storage (12 days). Nishimoto *et al.* (1985) proposed the value of 3.0 mg malonaldehyde/kg sample for good quality fish.

#### **4.1.7 Changes in protein patterns**

Protein patterns of whole and decapitated black tiger shrimp and white shrimp kept in ice are shown in Figure 29 and 30. The different protein patterns were observed between black tiger shrimp and white shrimp meats. Protein patterns of muscle proteins in black tiger shrimp remained unchanged during iced storage of 12 days (Figure 29). Nevertheless, the decrease in band intensity of MHC and actin was noticeable in whole white shrimp kept in ice for 12 days (Figure 30). This result corresponded with the substantial increase in TCA-soluble peptides of whole white shrimp at day 12 (Figure 31B). For decapitated white shrimp, only slight decrease in MHC was found. Therefore, pretreatment of shrimp by decapitation can be another means to retard the deterioration caused by proteolysis. Proteolytic degradation of other cytosolic

and cytoskeletal proteins present in the muscle caused by microbial growth and structural disintegration also occurred during ice storage of fish (*Priacanthus tayenus* and *P. macracanthus*) (Benjakul *et al.*, 2002). MHC was susceptible to proteolytic degradation than other muscle proteins, e.g. actin, troponin and tropomyosin (Benjakul *et al.*, 1997). From the result, icing methods did not show the pronounced effect on protein patterns of black tiger shrimp stored in ice.

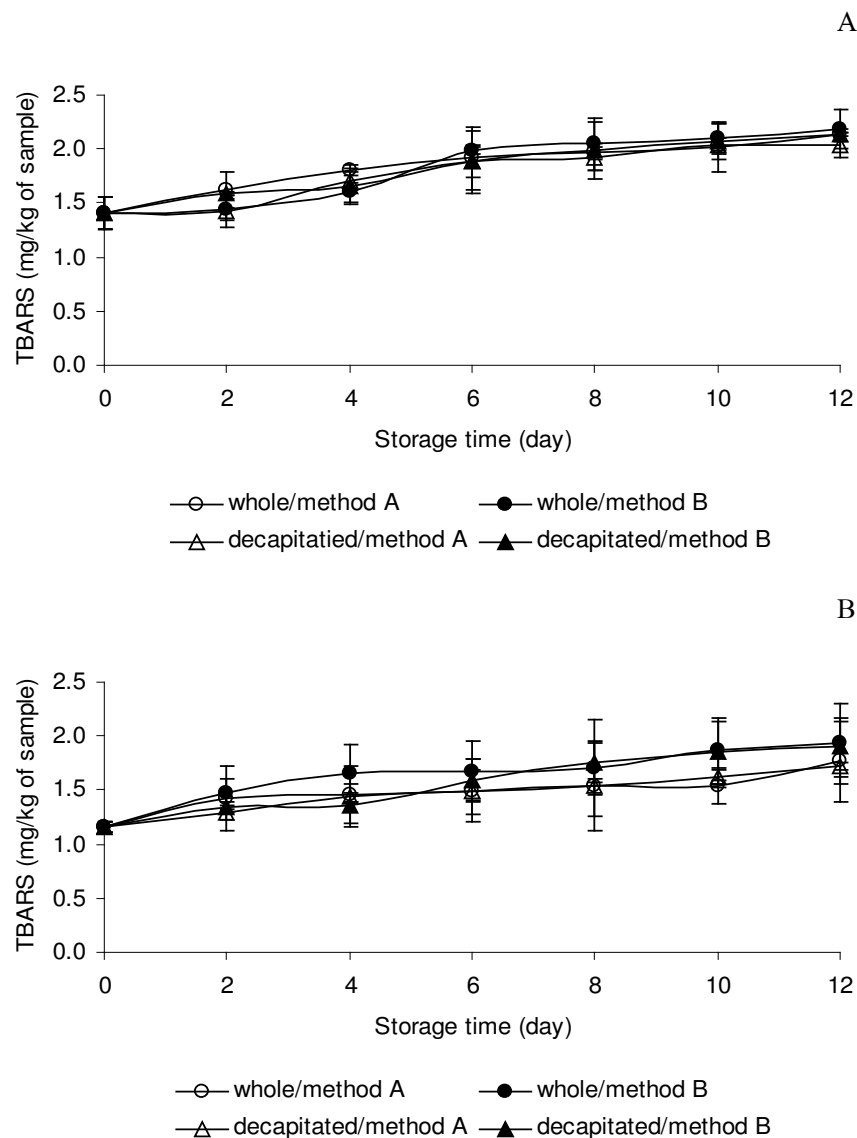


Figure 28 Changes in TBARS contents of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations.

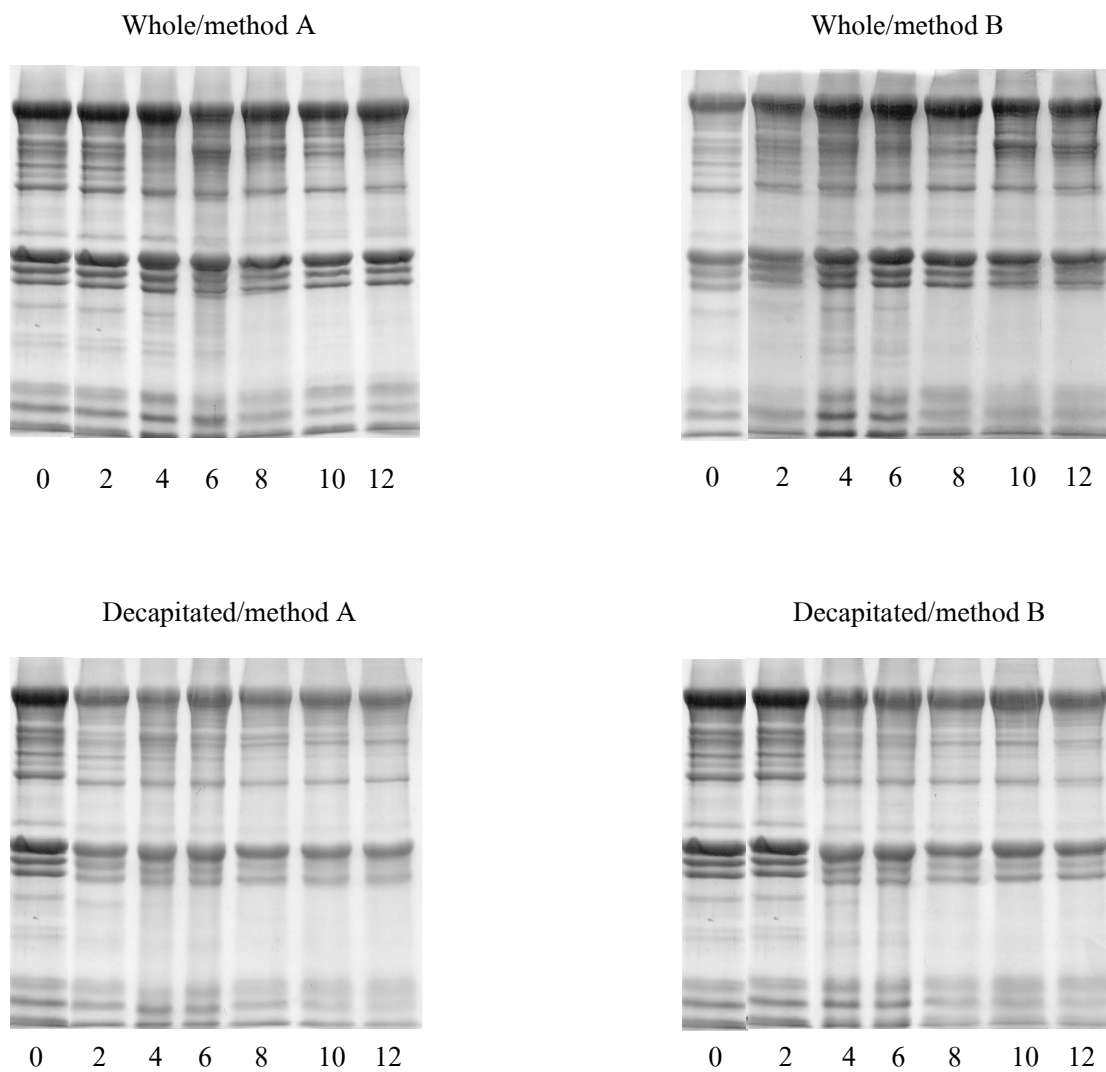


Figure 29 SDS-PAGE patterns of whole and decapitated black tiger shrimp with different icing methods during the storage. Numbers designate the storage time (days).

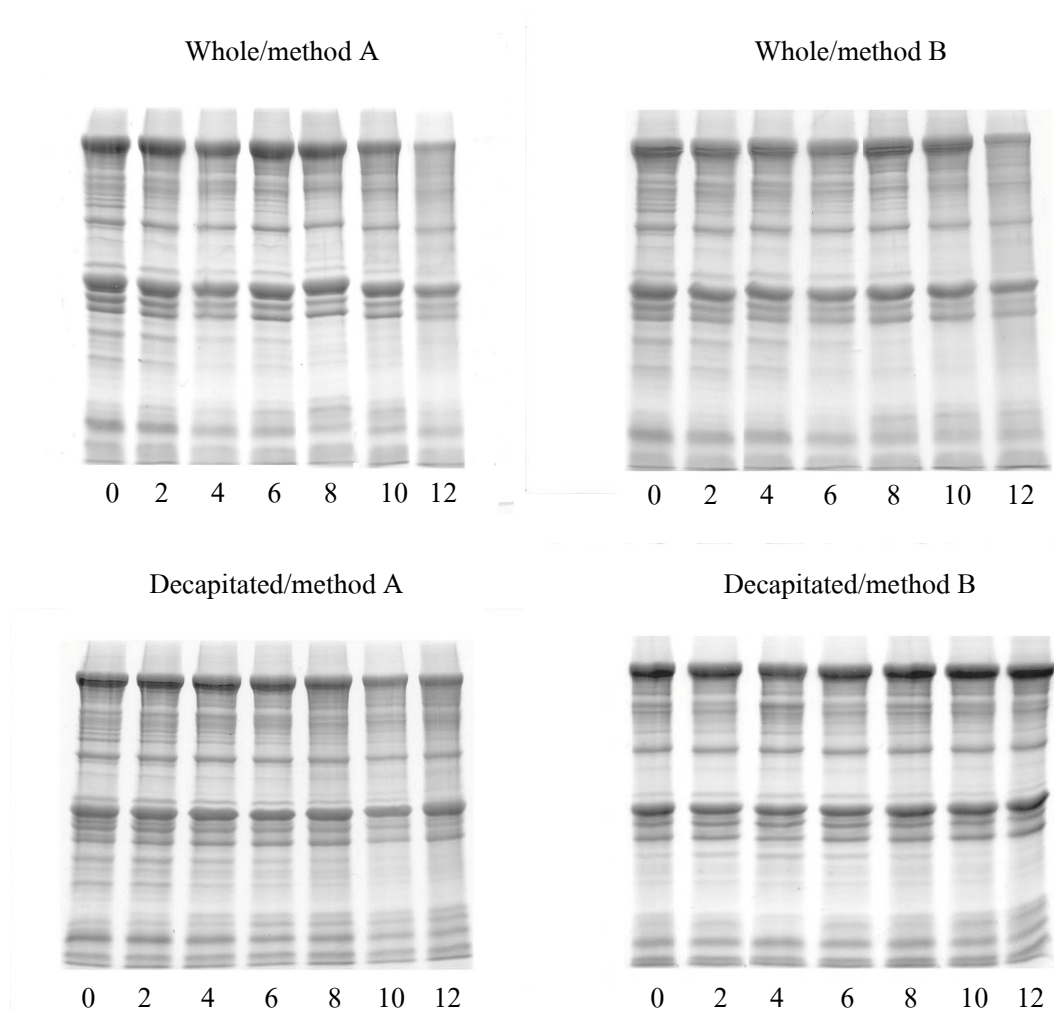


Figure 30 SDS-PAGE patterns of whole and decapitated white shrimp with different icing methods during the storage. Numbers designate the storage time (days).

#### 4.1.8 Changes in TCA-soluble peptide

TCA-soluble peptide contents of black tiger shrimp and white shrimp during iced storage are shown in Figure 31A and 31B, respectively. At day 0, black tiger shrimp meat contained slightly higher TCA-soluble peptide content than did white shrimp meat. This was possibly caused by the differences in small peptides or free amino acids presented in both species. After 2 days of storage, the marked increases in TCA-soluble peptide were observed for whole shrimps ( $p < 0.05$ ) but only slight increases were noticeable in the decapitated shrimps. From the result, slight decreases in TCA-soluble peptide were found after 8 days of storage.

The peptides generated might be leaked out together with molten ice, leading to the lower TCA-soluble peptide content. Water uptake of shrimp during storage in ice also contributed to the dilution of TCA-soluble peptides in shrimp muscles. The increase in TCA-soluble peptide in the first 8 days was in agreement with the increase in TVB (Figure 24). From the result, white shrimp generally showed the higher increase in TCA-soluble peptide, compared with black tiger shrimp. At the same storage period, TCA-soluble peptides of whole shrimp of both species were generally higher than those of decapitated shrimps throughout the storage ( $p < 0.05$ ). The result suggested that whole shrimp might contain higher activity of proteinases and a higher number of bacteria. Therefore, decapitated shrimp had the lower protein degradation caused by digestive and microbial proteinases. At the beginning of storage in ice, endogenous enzymes are mainly involved in the gradual loss of fish freshness. Thereafter, bacterial metabolism predominates and leads to final spoilage (Pacheco-Aguilar *et al.*, 2000). After 10 days of storage, the slight decrease in TCA-soluble peptide was found in all samples of both shrimps.

The results suggested that the decapitation of shrimp and low-temperature storage could lower the activity of endogenous autolytic enzymes in muscle as well as microbial proteinases. The hepatopancreas and digestive tract of crustaceans are very rich in proteolytic and collagenolytic enzyme (Tsai *et al.*, 1986). From the result, no marked effects of icing methods were noticeable on TCA-soluble peptides of both shrimps.

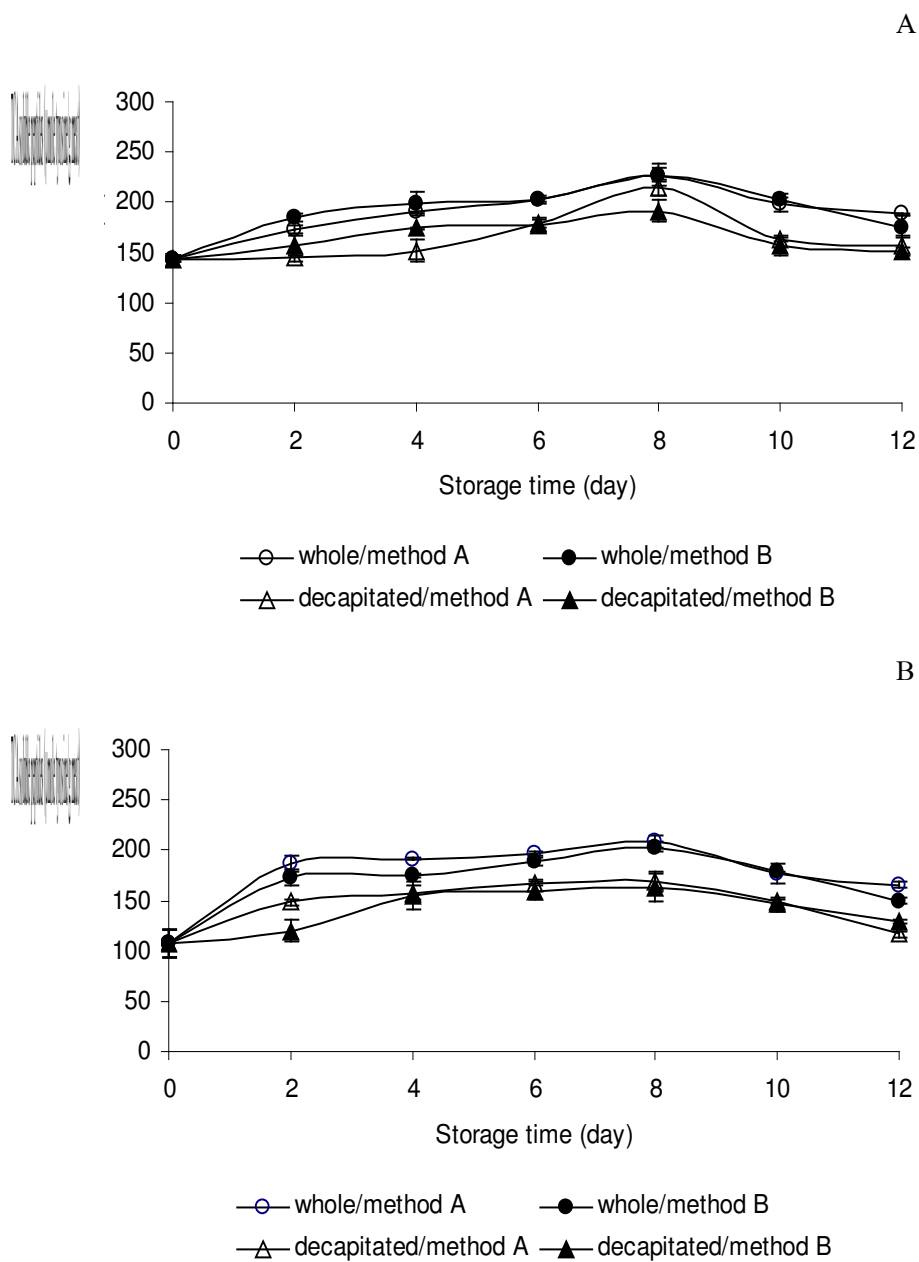


Figure 31 Changes in TCA-soluble peptides of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations.

## 4.2 Physical changes of black tiger shrimp and white shrimp during iced storage

### 4.2.1 Changes in shear force



Shear force of whole and decapitated black tiger shrimp and white shrimp during iced storage, both raw and cooked, are shown in Figure 32 and 33, respectively. Generally, shear force of raw white shrimp slightly decreased when storage time increased up to 12 days ( $p < 0.05$ ). Nevertheless, no changes in shear force were observed in raw black tiger shrimp meat during storage ( $p > 0.05$ ). From the results, it was suggested that shear force affected by storage time was dependent upon species. Thus, it was most likely that the destruction of muscle fibers of white shrimp was more pronounced, compared with black tiger shrimp. The degradation of shrimp tissue caused by hepatopancreatic enzymes started from the perimysium, endomysium, the Z line and the H zones with concurrent degradation of the connective fibers and the sarcoplasm (Nip *et al.*, 1985). Raw fish meat from most fish species softens after few days of chilled storage (Sato *et al.*, 1991). At the end of the storage (12 days), shear force of whole black tiger shrimp stored in ice with method A was lowest, when compared with other samples. This result suggested the role of collagenase localized in cephalothorax in hydrolysis of collagenous constituents in whole black tiger shrimp.

When black tiger shrimp and white shrimp meat was subjected to cooking, the former had the decrease in shear force, whereas the latter showed the increase in shear force as the storage time increased ( $p < 0.05$ ). After 12 days of storage, no differences in shear force of cooked white shrimp were observed ( $p > 0.05$ ) (Figure 33B). Nevertheless, the higher shear force was found in cooked decapitated black tiger shrimp regardless of icing methods, compared with cooked white shrimp. The increases in shear force of white shrimp stored in ice for a longer time might be associated with the losses of water caused by heating and shrinkage of muscle fibers. Therefore, the muscle bundles were more resistant to shearing. On the other hand, the decrease in shear force of black tiger shrimp kept in ice for a long time might be caused by the destruction of muscle fibers caused by the intensive hydrolysis of connective tissues.

#### **4.2.2 Changes in water holding capacity (WHC)**

WHC of whole and decapitated black tiger shrimp and white shrimp during iced storage is shown in Figure 34. Both shrimps had similar WHC. The continuous decreases in WHC of both species were observed throughout the storage ( $p < 0.05$ ). Black tiger shrimp and white shrimp had WHC with a range of 91-99% during iced storage. Hultman and Rustad (2002) reported that WHC of fresh Atlantic salmon ranged from of 93 to 91% during iced storage. The

result was in agreement with Zeng *et al.* (2005) who found the decrease in WHC of shrimp (*Pandalus borealis*) during storage in ice up to 6 days. WHC has been reported to be influenced by a number of factors including ultimate pH, protein denaturation, intra- and interfascicular spacing and sarcomere lengths (Offer and Knight, 1988).

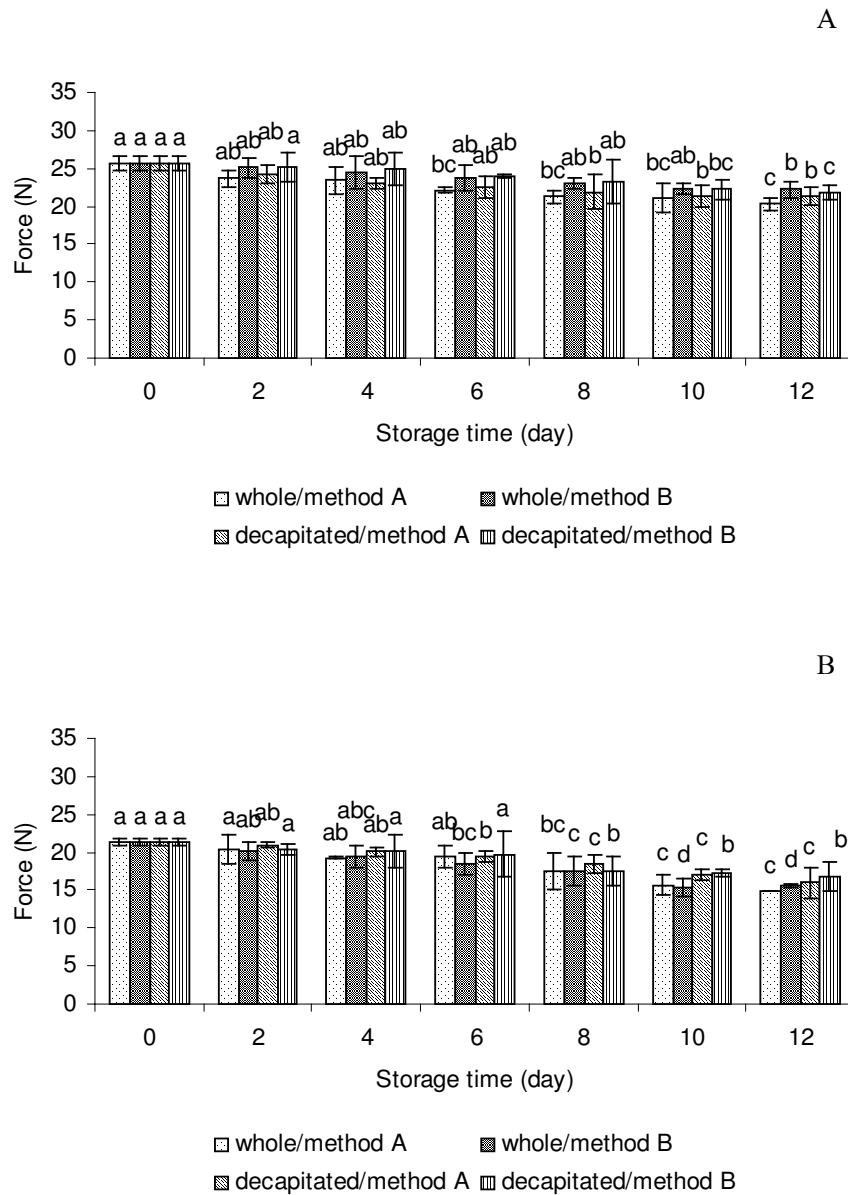


Figure 32 Changes in shear force of raw whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the

same treatment indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.

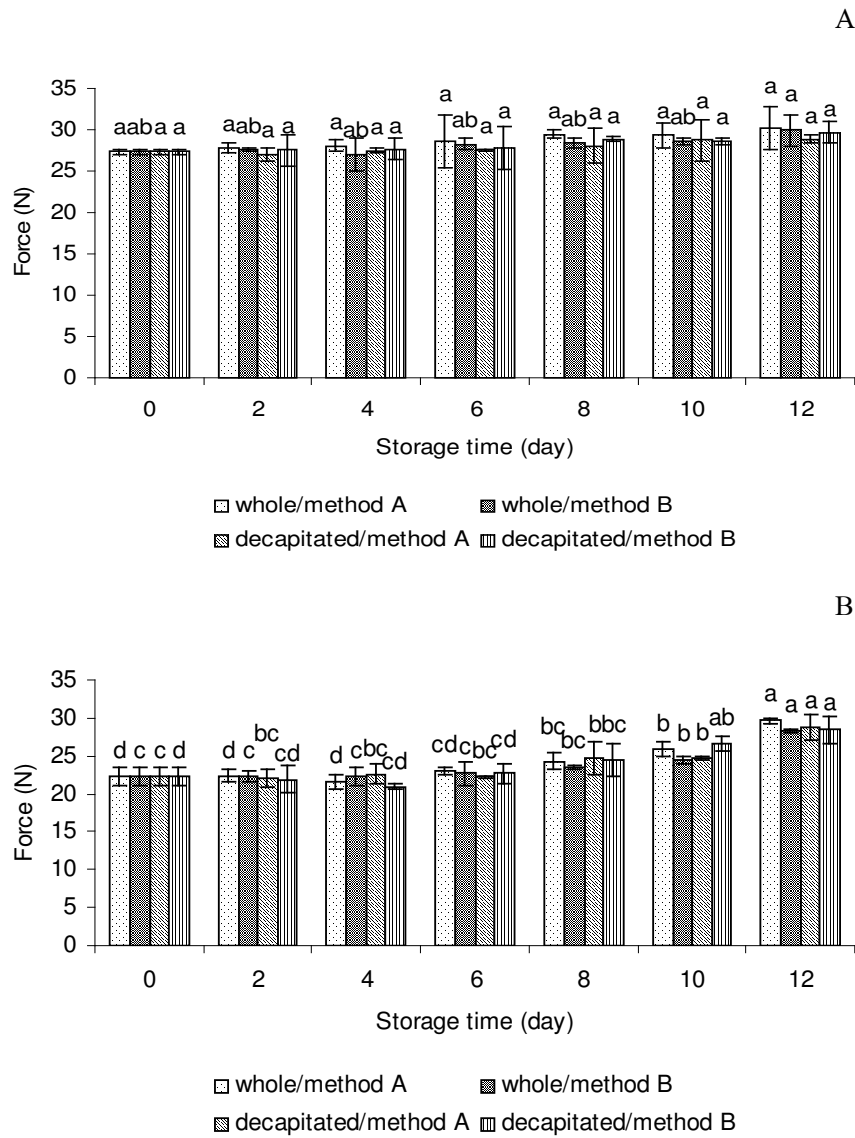


Figure 33 Changes in shear force of cooked whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the same treatment indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.

The lowered WHC of both shrimps stored in ice for a longer time might be due to the destruction of proteins, in which the hydrophobic portions 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. From the result, whole black tiger shrimp and white shrimp tended to have the lower WHC than the decapitated samples, except at day 10 when black tiger shrimp showed the higher WHC than the decapitated samples. Proteolytic enzymes in cephalothorax might induce the degradation of proteins, leading to the lowered ability to hold water of muscles. However, icing methods showed no effect on WHC of both shrimps, except for white shrimp kept in ice for 12 days, which had the lower WHC when stored in ice with method A.

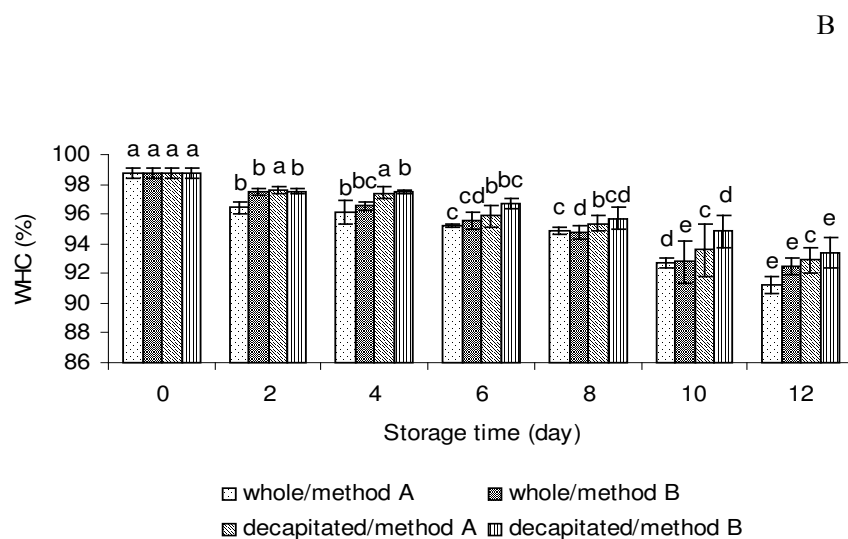
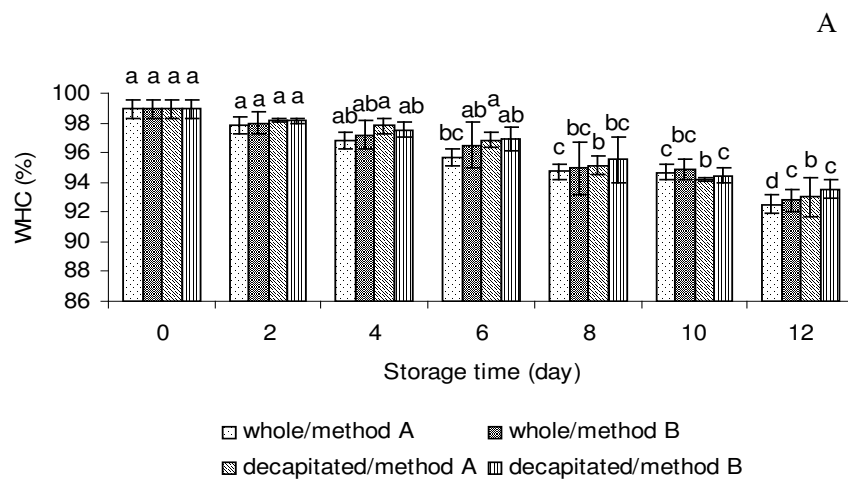


Figure 34 Changes in WHC of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the same treatment indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.

#### 4.2.3 Changes in melanosis score

Changes in melanosis score or black spot of whole and decapitated black tiger shrimp and white shrimp during iced storage are shown in Figure 35A and 35B, respectively. During the first 2 days of storage, no melanosis was found in both shrimps. This might result from the low temperature of storage, which could reduce the activity of polyphenol oxidase (PPO). Melanosis occurs in shellfish during storage as a result of the action of polyphenol oxidase (PPO) on tyrosine or its derivatives, such as tyramine, to form melanin (Rolle *et al.*, 1991). Melanosis of black tiger shrimp and white shrimp was observed at day 4 of storage. Melanosis took place initially at the ventral part of cephalothorax of these samples. No severe melanosis was found in the decapitated samples. Melanosis was also detected on pleopods, but not on clawed legs, telson, and tail carapace. Melanosis score of both shrimps increased as storage time increased ( $p<0.05$ ). At the same storage time, sample kept in ice with method A showed the lower melanosis score, compared with those stored in ice with method B ( $p<0.05$ ). When the ice was changed every 2 days, the molten ice was retained in the container. As a consequence, shrimps were immersed in the molten ice and oxygen was partially protected to involve in melanosis reaction.

Rolle *et al.* (1991) reported that tyramine may become the primary substrate postmortem for PPO catalyzed melanosis with a rate of oxidation 11-fold greater than tyrosine. With the same treatment, higher increases in melanosis score were found in white shrimp, compared with black tiger shrimp ( $p<0.05$ ). This might be associated with the differences in PPO activity between both species. Martinez-Alvarez *et al.* (2007) reported that Norway lobsters rapidly developed black spots or melanosis during iced storage (12 days). Montero *et al.* (2001) reported that the melanotic reaction begins at the head of chilled shrimps, and then spreads to the tail; the rate of spread of melanosis differs among the various species.

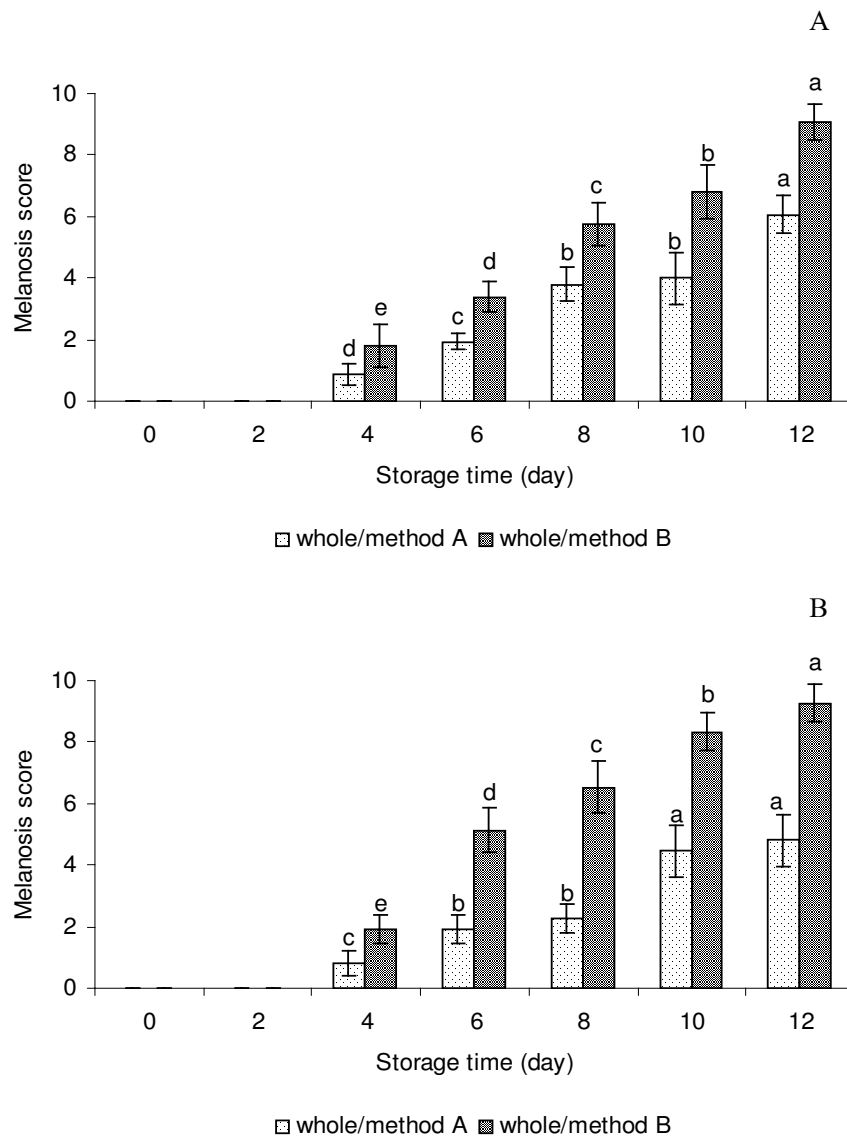


Figure 35 Changes in melanosis score of whole black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the same treatment indicate non significant differences ( $p > 0.05$ ). Bars represent the standard deviation from triplicate determinations.

#### 4.2.4 Changes in cooking loss

Cooking loss of black tiger shrimp and white shrimp stored in ice for different times is shown in Figure 36A and 36B, respectively. Cooking loss of both shrimps was more pronounced as storage time increased ( $p>0.05$ ). The amount of water bound by a tissue system decreases with heating, especially at higher temperature (Palka and Daun, 1999). Heating or cooking caused the denaturation of muscle proteins, leading to the losses in binding with proteins. At the same storage time, higher cooking losses were found in white shrimps samples, compared with black tiger shrimp ( $p<0.05$ ). The difference might be related to the different susceptibility of muscle protein toward heating as well as the different degree of muscle shrinkage. During thermal processing, shrimp proteins denature and lose their water holding ability, which can be accompanied by yield loss and dimensional changes (Erdogdu *et al.*, 2001). The losses in water might be associated with the tougher texture of shrimp. From the result, cooking loss was likely greater in whole shrimp, particularly after storage for more than 10 days. More degradation of protein and higher destruction of muscle structures in whole shrimp kept for extended time led to the enhanced losses in water when the shrimps were cooked. Icing methods generally had no effect on cooking losses of both shrimps at all storage times studied. The increases in cooking loss were coincidental with the decrease in water holding capacity (Figure 34) of both shrimps stored in ice for a longer time.

#### 4.2.5 Changes in L\*, a\* and b\*-values

Changes in L\*, a\* and b\*-values of raw black tiger shrimp and white shrimp during iced storage are shown in Table 16, 17 and 18, respectively. L\*, a\* and b\*-values gradually increased as the storage time increased ( $p<0.05$ ). L\*-value of black tiger shrimp was slightly higher than that of white shrimp. Black tiger shrimp had the lower a\* and b\*-values than white shrimp. Increases in lightness might be caused by the increase in water released to the surface as well as the denaturation of muscle proteins. Lightening is due to an increased light reflection, arising from light scattering by denatured proteins (Young and West, 2001). During extended iced storage, slight differences in color of both shrimps with different treatments were found. This might be associated with the changes in pigments in both shrimps as well as the denaturation of muscle proteins. The increases in a\* and b\*-values of both shrimps were possibly caused by more appearance of free pigments, mainly astaxanthin, due to the protein degradation or denaturation. Normally, astaxanthin was likely present as the carotenoproteins (Okada *et al.*,

1994). Body color is one of the major factors determining quality and price of shrimp (Boonyaratpalin *et al.*, 2001).

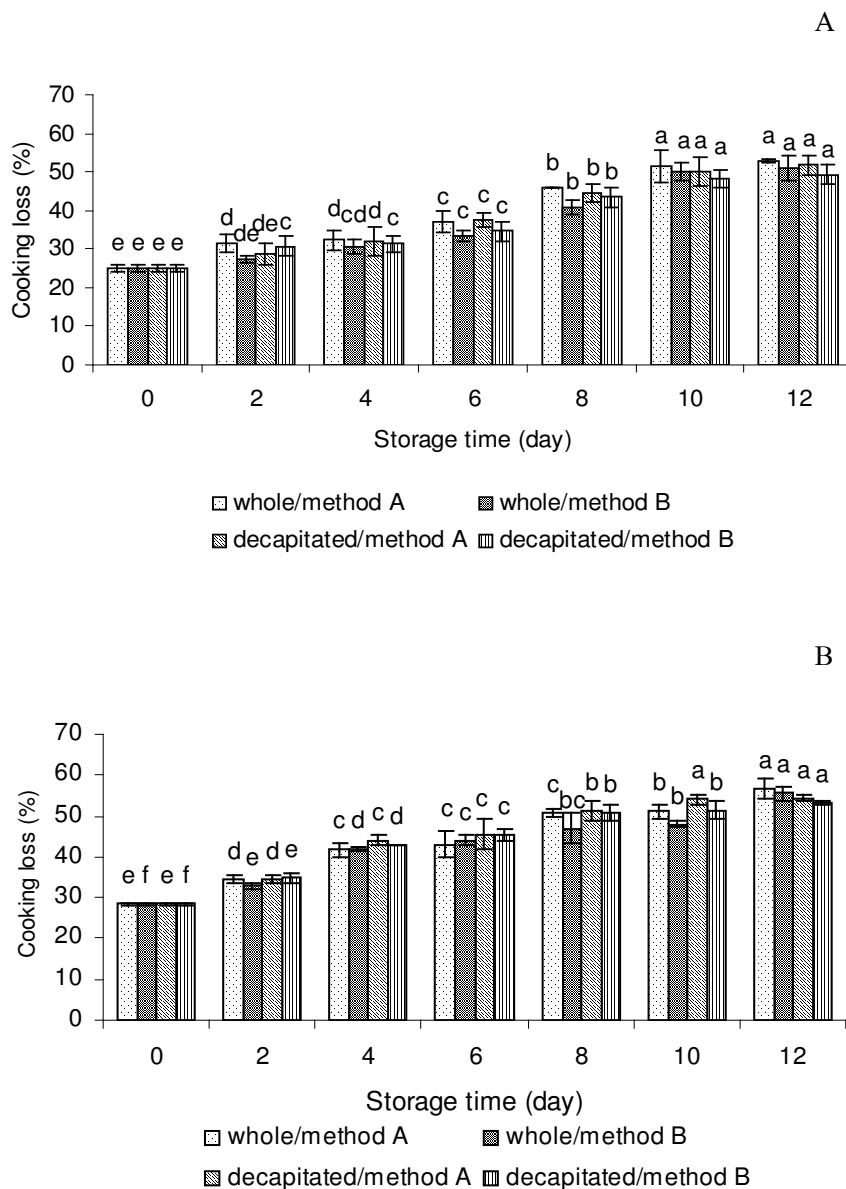


Figure 36 Changes in cooking loss of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. The same letters within the



same treatment indicate non significant differences ( $p>0.05$ ). Bars represent the standard deviation from triplicate determinations.

Table 16 Changes in L\*-value of raw meat of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	38.42±1.04d	39.44±0.65cd	41.56±2.32c	48.78±1.78b	51.26±0.75a	52.79±0.45a	51.18±1.89a
	Whole/method B	38.42±1.04e	41.84±1.42d	44.66±0.68c	47.04±1.21b	49.21±0.08a	49.85±2.03a	50.85±0.71a
	Decapitated/method A	38.42±1.04c	40.54±2.69bc	41.94±1.63b	48.93±1.38a	48.84±1.88a	50.45±2.03a	51.44±1.21a
	Decapitated/method B	38.42±1.04d	42.06±0.29c	43.46±2.05c	49.00±1.35b	48.44±0.77b	50.90±1.97ab	52.08±0.29a
White shrimp	Whole/method A	36.72±0.69e	45.10±0.18d	48.51±0.96c	50.02±0.19c	51.83±1.45b	53.51±0.40a	52.92±1.10a
	Whole/method B	36.72±0.69e	45.00±0.32d	47.70±0.13c	49.92±0.90c	50.60±0.72b	49.78±0.39b	52.81±0.99a
	Decapitated/method A	36.72±0.69e	43.77±1.29d	47.67±0.23c	47.67±0.23c	50.32±0.76b	51.40±2.01b	54.90±0.84a
	Decapitated/method B	36.72±0.69e	42.18±1.14de	47.63±0.68c	48.73±0.69c	51.21±0.34b	52.00±0.97a	52.82±0.53a

Values are given as mean  $\pm$ SD from triplicate determinations. Different letters in the same row indicate significant differences ( $p<0.05$ ).

Table 17 Changes in a\*-value of raw meat of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	-2.68±0.37c	-2.27±0.37bc	-1.89±0.43bc	-1.87±0.47bc	-1.63±0.28ab	-1.45±0.34ab	-1.00±0.09a
	Whole/method B	-2.68±0.37c	-2.65±0.17c	-2.01±0.38b	-1.96±0.10b	-1.87±0.27b	-1.72±0.45b	-1.05±0.24a
	Decapitated/method A	-2.68±0.37c	-2.65±0.13c	-2.57±0.23c	-2.13±0.15c	-1.99±0.29bc	-0.88±0.27ab	-0.52±0.13a
	Decapitated/method B	-2.68±0.37b	-2.63±0.15ab	-2.62±0.25ab	-2.52±0.27ab	-2.27±0.33ab	-2.34±0.43ab	-2.04±0.24a
White shrimp	Whole/method A	-1.65±0.40d	-1.51±0.31bc	-1.45±0.31b	-1.10±0.44a	-0.34±0.00a	-0.21±0.06a	0.30±0.06a
	Whole/method B	-1.65±0.40c	-1.30±0.24b	-1.59±0.37ab	-1.36±0.50a	-0.95±0.44a	-0.54±0.13a	0.31±0.07a
	Decapitated/method A	-1.65±0.40c	-1.48±0.47c	-1.32±0.19c	-1.17±0.14ab	-0.66±0.34ab	-0.71±0.26a	-0.65±0.09a
	Decapitated/method B	-1.65±0.40b	-1.63±0.21b	-1.59±0.01b	-1.05±0.23b	-0.90±0.14a	-1.01±0.11a	-0.88±0.05a

Values are given as mean  $\pm$ SD from triplicate determinations. Different letters in the same row indicate significant differences ( $p<0.05$ ).

Table 18 Changes in b\*-value of raw meat of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	-3.77±0.69c	-3.50±0.43c	-3.04±0.48bc	-2.80±0.77bc	-2.33± 0.13b	-1.18±0.10a	-1.16±0.36a
	Whole/method B	-3.77±0.69c	-3.02±0.79bc	-2.91±0.24bc	-2.74±0.08bc	-2.19± 0.53b	-0.78±0.17a	-0.58±0.15a
	Decapitated/method A	-3.77±0.69c	-3.62±0.20c	-2.78±0.81bc	-2.55±0.25b	-2.54±0.33b	-1.08±0.17a	-0.65±0.16a
	Decapitated/method B	-3.77±0.69b	-3.74±0.28b	-3.70±0.24b	-2.96±0.50b	-2.96±0.50b	-1.67±0.33a	-1.11±0.30a
White shrimp	Whole/method A	-0.94±0.22c	-0.57±0.17c	-0.58±0.15c	0.31±0.09b	2.57±0.64ab	2.68±0.63ab	2.92±0.46a
	Whole/method B	-0.94±0.22c	-0.78±0.06c	-0.08±0.08c	1.52±0.32c	3.17±0.37b	2.85±0.64ab	3.31±0.37a
	Decapitated/method A	-0.94±0.22f	0.43±0.17ef	0.75±0.19de	1.47±0.22cd	1.90±0.10bc	2.41±0.36b	2.83±0.38a
	Decapitated/method B	-0.94±0.22d	-0.88±0.29d	0.34±0.11cd	0.92±0.20bc	1.69±0.36b	2.17±0.75	1.94±0.63a

Values are given as mean  $\pm$ SD from triplicate determinations. Different letters in the same row indicate significant differences ( $p < 0.05$ ).

L\*, a\* and b\*-values of cooked black tiger shrimp and white shrimp stored in ice for different times are shown in Table 19, 20 and 21, respectively. The marked increases in L\*, a\* and b\*-values of both shrimps were found after cooking, compared with the fresh samples (Table 16, 17 and 18). Black tiger shrimp had the much higher a\*-value than white shrimp. The slight decreases in L\*-value of both shrimps were observed when the storage time increased (Table 19). The decreases in L\*-value in sample stored for a longer time might be associated with the increases in melanosis as well as non-enzymatic browning occurred during the extended iced storage. The increases in a\* and b\*-values of cooked samples of both species were noticeable as the storage time increased. Hydrolyzed muscle proteins observed in shrimp stored for a longer time might not be able to bind with astaxanthin. After heating, those proteins underwent denaturation easily and the redness of astaxanthin can be apparently observed. Concentration of astaxanthin is the main factor controlling shrimp body color (Stepnowski *et al.*, 2004).

Table 19 Changes in L\*-value of cooked meat of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	67.61±0.25a	66.27±0.25a	66.94±1.48a	66.08±1.13a	63.25±2.07b	60.64±1.92c	58.29±1.12c
	Whole/method B	67.61±0.25a	65.33±0.57b	64.80±1.43bc	64.53±0.73bc	63.20±0.94cd	62.32±1.33de	61.15±1.55e
	Decapitated/method A	67.61±0.25a	65.87±0.55ab	65.19±1.05ab	65.05±2.51ab	65.18±0.84ab	63.58±2.25b	59.67±1.25c
	Decapitated/method B	67.61±0.25a	65.32±0.17b	64.59±0.29b	64.73±0.75b	63.17±0.28c	62.84±0.49c	60.74±1.58d
White shrimp	Whole/method A	73.03±0.55a	69.90±0.71b	69.83±1.98b	66.10±0.59c	66.29±1.22c	64.83±1.13c	65.67±2.41c
	Whole/method B	73.03±0.55a	72.40±0.62a	69.88±1.03b	66.81±1.20c	66.53±0.28c	64.83±0.44d	63.02±0.64e
	Decapitated/method A	73.03±0.55a	71.33±0.66b	71.04±0.42b	68.33±0.57c	68.23±1.28c	67.50±0.43d	67.03±0.18d
	Decapitated/method B	73.03±0.55a	69.33±0.11b	69.44±0.75b	69.61±0.66b	67.03±0.75c	67.28±1.19c	67.17±0.13c

Values are given as mean  $\pm$ SD from triplicate determinations. Different letters in the same row indicate significant differences ( $p < 0.05$ ).

Table 20 Changes in a\*-value of cooked meat of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	15.24±2.25a	17.23±0.25a	18.50±0.38a	18.32±2.09a	17.67±2.40a	17.16±1.05a	17.22±2.74a
	Whole/method B	15.24±2.25c	16.68±0.76bc	19.57±0.16a	18.31±0.50ab	17.94±1.14ab	17.47±1.02ab	17.49±0.96c
	Decapitated/method A	15.24±2.25c	16.90±1.89bc	20.93±2.63a	19.85±0.20ab	19.60±0.18ab	19.32±0.46ab	18.07±1.12abc
	Decapitated/method B	15.24±2.25b	18.11±0.58a	19.57±0.78a	18.73±0.39a	18.47±1.72a	18.04±1.84a	17.62±1.10ab
White shrimp	Whole/method A	9.55±0.78c	11.24±1.91b	12.45±0.35a	11.97±0.75ab	11.86±0.10ab	11.87±0.35ab	10.33±1.54b
	Whole/method B	9.55±0.78a	9.93±0.84a	11.99±0.83a	11.44±1.14a	11.13±0.14a	10.97±1.79a	10.82±2.69a
	Decapitated/method A	9.55±0.78b	12.04±2.36ab	13.35±0.33a	11.33±0.59ab	10.94±2.30ab	10.97±0.20ab	10.58±0.63b
	Decapitated/method B	9.55±0.78c	11.24±2.48ab	12.50±0.47a	10.44±0.85abc	11.60±0.27ab	12.18±1.31ab	11.08±0.28ab

Values are given as mean  $\pm$ SD from triplicate determinations. Different letters in the same row indicate significant differences ( $p < 0.05$ ).

Table 21 Changes in b\*-value of cooked meat of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	19.40±1.51e	20.00±0.25e	21.23±0.35de	22.46±1.23cd	23.85±0.30bc	24.57±1.43ab	26.09±1.05a
	Whole/method B	19.40±1.51d	20.27±1.55cd	20.67±0.26cd	21.76±0.39bc	22.80±0.78ab	23.14±1.05ab	24.22±0.72a
	Decapitated/method A	19.40±1.51c	21.10±0.22bc	20.49±2.62bc	21.51±0.47bc	22.83±1.29ab	23.05±1.00ab	24.12±1.01a
	Decapitated/method B	19.40±1.51c	21.88±0.69b	21.65±0.23ab	22.95±1.53ab	23.25±1.21ab	23.39±1.32ab	24.04±0.83a
White shrimp	Whole/method A	16.34±0.46a	16.27±1.18a	16.58±1.70a	17.48±0.46a	16.17±0.79ab	17.04±0.75a	17.92±1.43ab
	Whole/method B	16.34±0.46bc	15.10±1.40c	16.20±1.72bc	16.21±0.81bc	16.18±0.07c	18.54±0.02a	17.73±0.75ab
	Decapitated/method A	16.34±0.46bc	15.56±0.50c	16.70±0.58b	18.46±0.98b	18.22±0.27a	17.05±0.78b	17.38±0.43ab
	Decapitated/method B	16.34±0.46b	17.28±1.38ab	16.99±0.15ab	16.01±2.29ab	17.19±0.74ab	18.26±0.98a	18.12±0.36a

Values are given as mean ±SD from triplicate determinations. Different letters in the same row indicate significant differences ( $p < 0.05$ ).

#### 4.3 Microbiological changes of black tiger shrimp and white shrimp during iced storage

##### 4.3.1 Changes in total viable count

Changes in TVC of black tiger shrimp and white shrimp during ice storage are depicted in Figure 37A and 37B, respectively. The differences in TVC of fresh shrimps between two species were observed. TVC of fresh black tiger shrimp and white shrimp were 4.43 and 2.91 log cfu/g, respectively. The different microflora between species was associated with different microbial population of waters, in which they lived (Liston, 1980). During the storage in ice, TVC of black tiger shrimp decreased by more than one log after 2 days of storage. Subsequently, TVC gradually increased up to 10 days. No marked changes in TVC were found between day 10 and 12. The initial reduction in TVC was mainly due to the effect of cold shock (Lakshmanan *et al.*, 2002). The differences in TVC of black tiger shrimp with different treatments were observed after 8 days of storage ( $p < 0.05$ ). At the end of storage (12 days), TVC of whole shrimp stored in ice with method A had the highest TVC, whereas the decapitated shrimp kept in ice with method

B contained the lowest TVC. For white shrimp, gradual increase in TVC was observed throughout the storage. Generally, no difference in TVC was noticeable among different treatments within the first 10 days of storage. However, the lowest TVC at the end of storage was found in the decapitated samples stored in ice with method B ( $p < 0.05$ ). The highest TVC of both species at day 12 was concomitant with the highest content of biogenic amines (Table 14 and 15). From the results, it was suggested that decapitation with and changing the ice every day during storage could retard the microbial growth. Biogenic amines are usually generated by microbial decarboxylation of specific free amino acids in fish or shellfish tissue (Rawles *et al.*, 1996). From the result, TVC exceeded  $10^5$  cfu/g after 10 days of storage, which is the limit for acceptability (ICMSF, 1986). It was noted that TVC determination was conducted at  $37^\circ\text{C}$  and the value represented mostly mesophilic bacteria. This might not indicate the microbial load in the shrimp stored at low temperature. Those psychrophilic microorganisms most likely played a role in spoilage of those shrimps.

#### 4.3.2 Changes in coliforms and *E. coli*

Changes in coliforms and *E. coli* of black tiger shrimp and white shrimp during iced storage are shown in Table 22 and 23. Coliforms were absent in fresh shrimp of both species (Table 22). However, Jeyasekaran *et al.* (2004) reported that fresh fish (*Lethrinus miniatus*) had an initial total coliforms count of 5.0 MPN/g. The gradual increases in coliforms during ice storage of both shrimps were observed until day 8 of storage time. Keeping fish at low temperatures reduced bacterial growth (Sasi *et al.*, 2000). Coliforms of both shrimps sharply increased after 8 days of storage. Enterobacteriaceae was found in shrimps (*Penaeus monodon*) on days 7 of iced storage (Rahaman, 2001). The highest increases in coliforms of both species were found in samples kept in ice with method A. Coliforms contaminated from the environment could reside in molten ice, which was retained in the container together with the shrimp. As a result, growth of coliforms was enhanced. At the end of storage, whole black tiger shrimp stored in ice with method A had the highest coliforms, while the decapitated white shrimp kept in ice with method A showed the highest coliforms. This result was in agreement with that of TVC.

Coliforms are the bacteria used as the sanitation index for fish and shellfish (Feng and Hartman, 1982). Buchanan (1991) reported that coliforms could be used as a better indicator of process integrity than TVC.

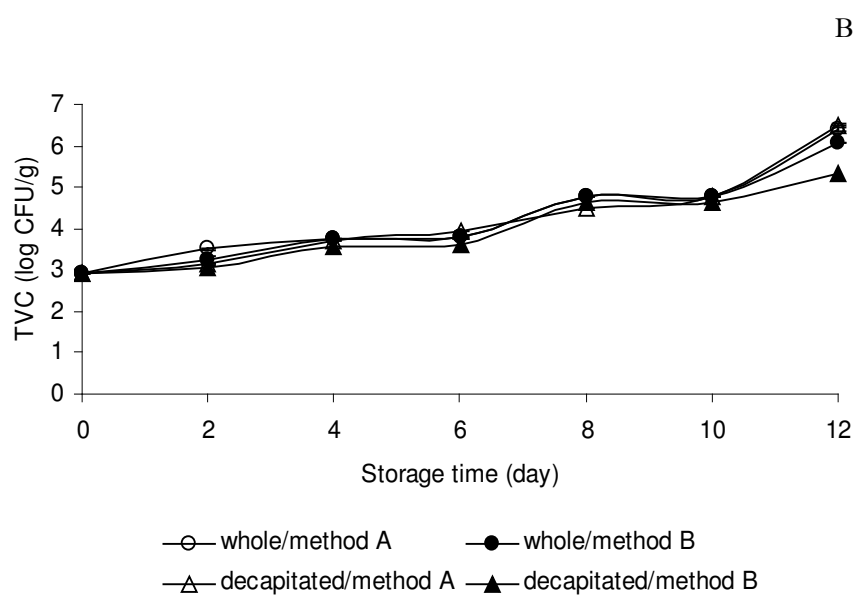
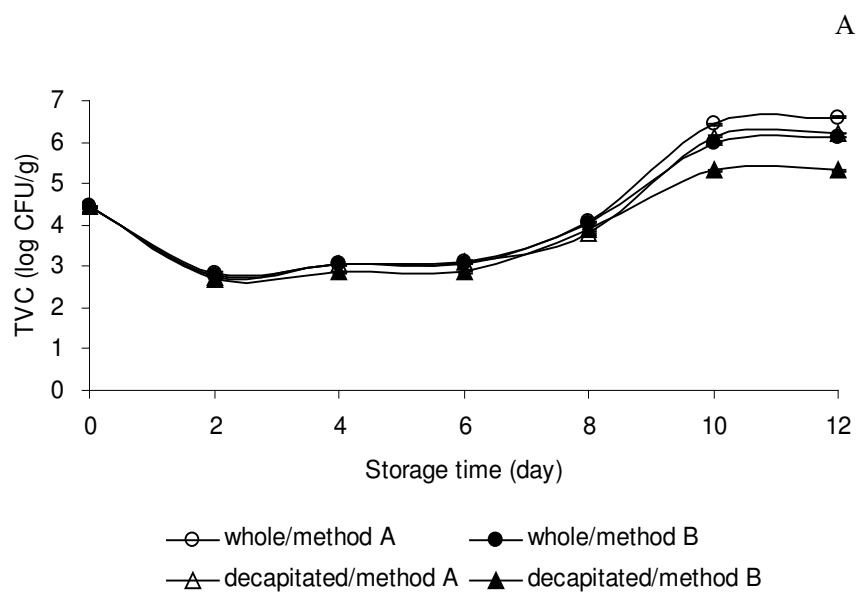


Figure 37 Changes in microbial load of whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations.

Table 22 Changes in coliforms (MPN/g) of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	< 3	4	21	23	40	150	210
	Whole/method B	< 3	3	9	11	40	70	90
	Decapitated/method A	< 3	3	9	9	23	90	150
	Decapitated/method B	< 3	4	15	15	40	90	90
White shrimp	Whole/method A	< 3	4	15	21	23	90	150
	Whole/method B	< 3	4	7	21	23	70	90
	Decapitated/method A	< 3	4	7	15	21	150	200
	Decapitated/method B	< 3	4	7	15	21	40	90

Changes in *E. coli* of both shrimps during iced storage are depicted in Table 23. No *E. coli* was detectable in both shrimps within the first 4 days of storage (Table 23). *E. coli* is a microbial indicator of fecal contamination from animal and human on food (Bredie and de Boer, 1992.). The absence of *E. coli* suggested no fecal contamination of the samples used. *E. coli* was found in all samples after 4 day of storage and gradually increased until the end of storage time. However, in this experiment, high TVC ( $10^3$  cfu/g), coliforms (9 MPN/g) and *E. coli* (4 NPN/g) were observed in ice used for chilling. *E. coli* found in black tiger shrimp and white shrimp was possibly contaminated from ice used for chilling. When microbial quality of ice used in this study was evaluated, high TVC, coliforms and *E. coli* were found. This could be an essential source of microorganisms. Bacteria belonging to the family Enterobacteriaceae were detected in a few

numbers in crustacean muscle during iced storage (Moori *et al.*, 1988). For both species, whole shrimps stored in ice with method A had the highest *E. coli*, followed by the decapitated shrimp kept in ice with method A. Thus, icing method seemed to affect *E. coli* load in shrimp stored in ice.

Table 23 Changes in *Escherichia coli* (MPN/g) of whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage

Species	Treatments	Storage time (day)						
		0	2	4	6	8	10	12
Black tiger shrimp	Whole/method A	< 3	< 3	< 3	9	15	21	23
	Whole/method B	< 3	< 3	< 3	4	11	14	14
	Decapitated/method A	< 3	< 3	< 3	4	11	15	21
	Decapitated/method B	< 3	< 3	< 3	7	11	11	14
White shrimp	Whole/method A	< 3	< 3	< 3	7	9	11	14
	Whole/method B	< 3	< 3	< 3	3	7	7	9
	Decapitated/method A	< 3	< 3	< 3	3	4	11	11
	Decapitated/method B	< 3	< 3	< 3	3	4	7	9

#### 4.4 Sensorial changes of black tiger shrimp and white shrimp during iced storage

Total quality scores of raw and cooked black tiger shrimp and white shrimp stored in ice for different times are shown in Figure 38. The total quality score of the raw samples of both species increased with increasing storage time, indicating the lowered quality. From the result, whole shrimp showed the higher score, compared with the decapitated shrimps. However, icing methods did not show the pronounced effect on the total quality score of both shrimps during storage. Yamagata and Low (1988) reported that the overall appearance of shrimp (*Penaeus merguensis*) was acceptable within the first 2 days of storage with slight blackening in the gill region. Whole shrimp of both species turned to be orange in color and blackening occurred in the cephalothorax of shrimps. This led to unacceptability of whole shrimp kept for a long time. For decapitated shrimps, all samples were unacceptable on 12 days of storage as



evidenced by the score lower than 5. Score above 5 is the limit for acceptability (U.S. FDA, 2005). For raw white shrimp, the increase in total quality in all treatments was observed after 2 days of storage ( $p < 0.05$ ), (Figure 38). Whole white shrimp showed the higher total quality than decapitated samples during iced storage. This indicated that decapitation could extend the shelf-life of both shrimps. Cephalothorax contained a high amount of enzymes, particularly PPO and proteinase, which likely showed the detrimental effect on shrimp quality.

Total quality of cooked black tiger shrimp and white shrimp during storage in ice are shown in Figure 38. The total quality score of cooked samples of both species increased with increasing storage time. No differences in total quality between treatments for both shrimps were observed throughout the storage of 12 days. Based on the score of 5 as the limit for acceptability, all shrimps were rejected after 12 days of storage.

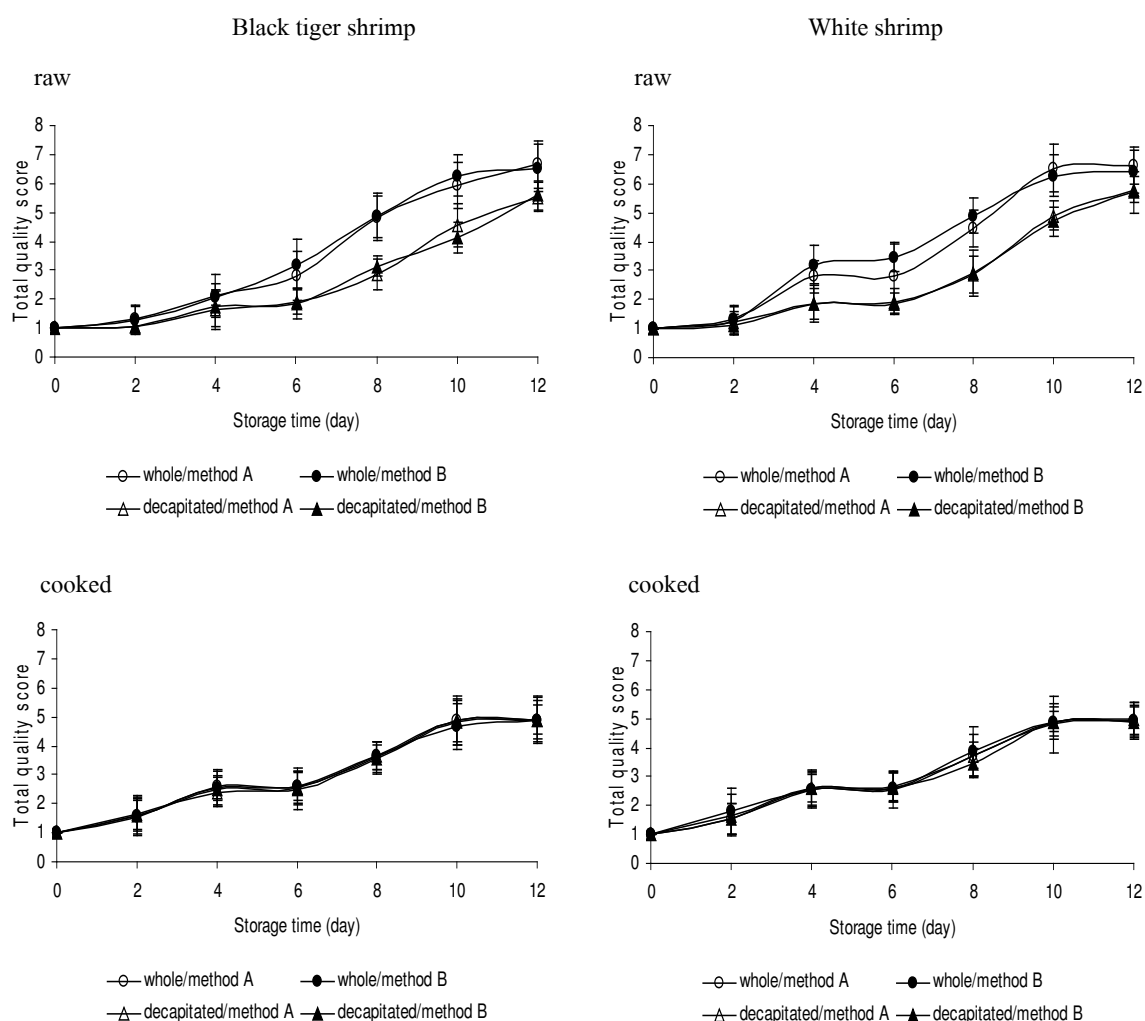


Figure 38 Changes in total quality of raw and cooked whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations. Score 1 = Match; Score 10 = Reject.

Changes in appearance score of raw and cooked black tiger shrimp and white shrimp stored in ice for various times are shown in Figure 39. The slight increase in appearance score were found in raw sample within the first 4 days of storage ( $p < 0.05$ ) (Figure 39). Thereafter, the marked increases were noticeable and the scores reached the plateau at day 10. For black tiger shrimp, decapitation resulted in the lower score of shrimp stored in ice with method A, particularly after 4 days of storage. However, the decapitation showed no pronounced effect on appearance score of white shrimps throughout the storage. Icing methods exhibited the influence on the appearance score of whole black tiger shrimp. Sample stored in ice with method B showed the higher appearance score than those with method A. The higher score indicated the lower acceptability of shrimp. Molten ice retaining in the container could submerge the sample. Thus, the shrimps were protected from direct contact with oxygen molecule, resulting in the lowered blackening. Fatima *et al.* (1988) reported that prime quality shrimp (*Penaeus merguensis*) could be obtained within 8 days on ice. The appearance score was concomitant with total quality of both shrimps.

The increases in appearance score of cooked black tiger and white shrimp were obtained with increasing storage time ( $p < 0.05$ ) as depicted in Figure 39. No differences in appearance score between treatments of cooked samples of both species. However, whole shrimps tended to have the higher appearance score, particularly when the storage time increased. Based on the appearance score, black tiger shrimps were acceptable up to 12 days. White shrimps kept for up to 12 days were also still acceptable.

Changes in texture score of raw and cooked black tiger shrimp and white shrimp are depicted in Figure 40. Generally, texture score of raw black tiger shrimp and white shrimp increased with increasing storage time ( $p < 0.05$ ). This suggested that postmortem autolysis occurred during the extended storage. Nevertheless, no changes in texture score in all samples were found after 10 days of storage ( $p > 0.05$ ). For white shrimp, the higher increase in texture score was noticeable in comparison with black tiger shrimps, especially after 6 days of storage in

ice. Generally, shrimps kept for a longer time had the soft texture. Shamshad *et al.* (1990) reported that the increase in proteolytic activity of shrimp (*Penaeus merguensis*) was noticed with increasing time of storage at 0°C. Also shrimp contained a lot of non-protein nitrogenous compounds, easily metabolized by microorganisms (Liston, 1982). This possibly enhanced the loss in texture of raw shrimps.

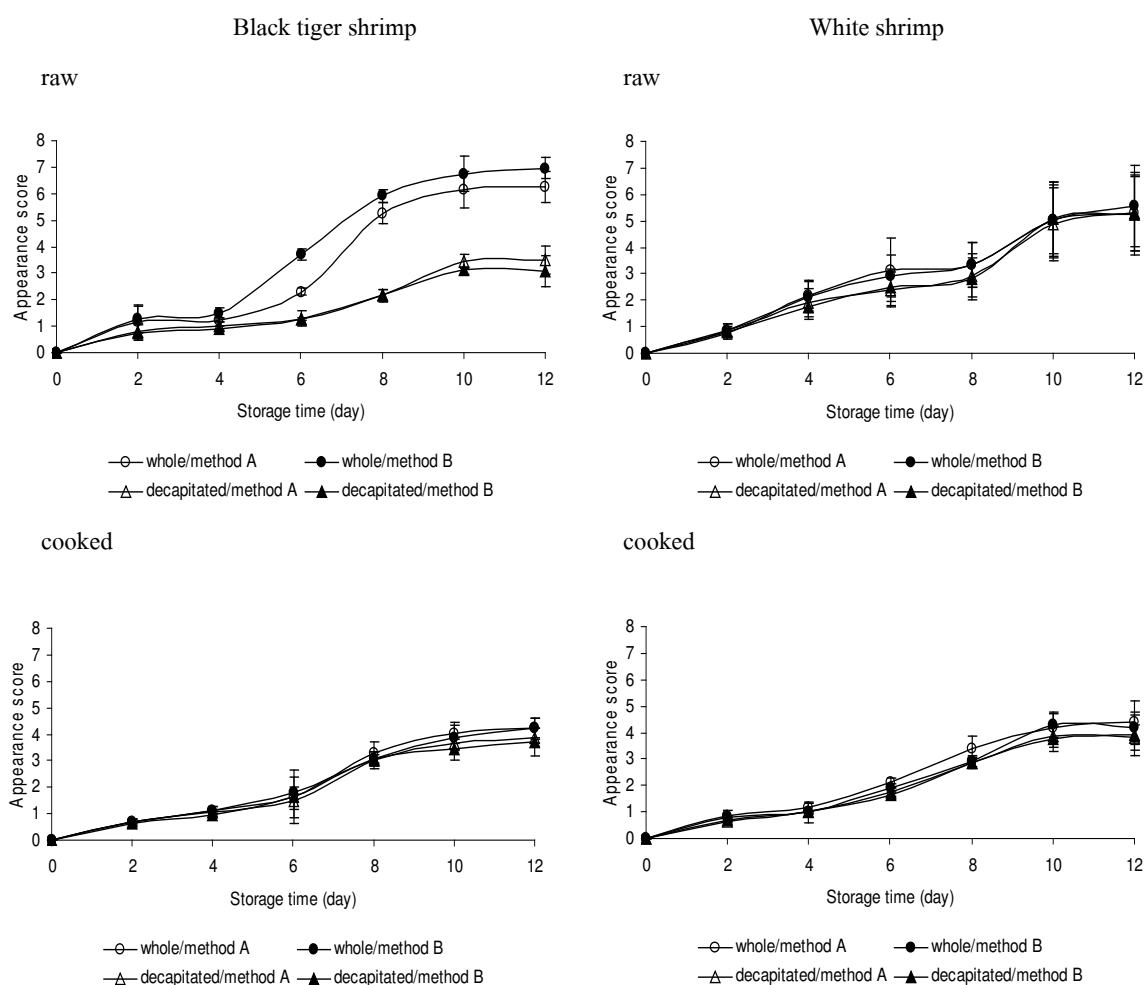


Figure 39 Changes in appearance score of raw and cooked whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations. Score 0 = Strongest Pass; Score 10 = Strongest Fail.

Texture scores of cooked black tiger shrimp and white shrimp stored in ice for different times are shown in Figure 40. Texture score in all samples increased with increasing storage time ( $p < 0.05$ ). No differences in texture score between all treatments of both species was observed when stored for up to 8 days ( $p > 0.05$ ) (Figure 40). However, the texture score tended to be higher in whole shrimps in comparison with decapitated shrimps at days 10 and 12. Icing the decapitated shrimps of both species with method B rendered the shrimp with the lowest score at day 12, indicating the highest acceptability of this sample. Based on the texture score, both shrimps were acceptable within 10 days in ice. When comparing the texture score between both species, it was found that cooked black tiger shrimp had slightly lower score than did white shrimp, particularly after 6 days of storage. Destruction of muscle fibers and the degradation of muscle proteins might be different between both species as evidenced by different shear force (Figure 32).

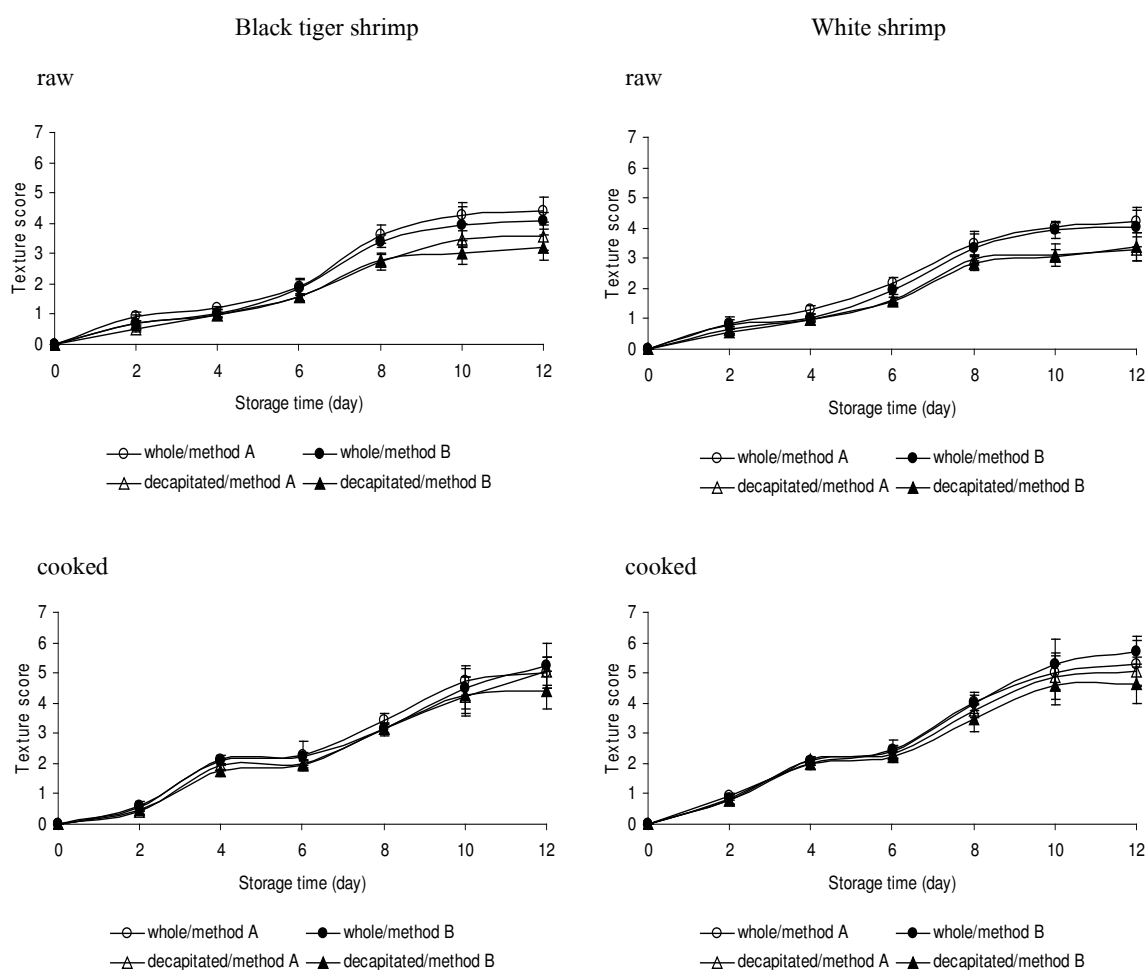


Figure 40 Changes in texture score of raw and cooked whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations. Score 0 = Strongest Pass; Score 10 = Strongest Fail.

Odor scores of raw and cooked samples of black tiger shrimp and white shrimp are depicted in Figure 41. Fresh shrimps used in this study exhibited fresh seaweedy odor characteristics. The gradual increases in odor score of raw samples of black tiger shrimp were found up to 8 days (Figure 41). Thereafter, the sharp increases in odor score were noticeable in all samples. Whole shrimps generally had the higher score than did decapitated shrimp after 8 days of storage. However, icing methods had no impact on odor score ( $p>0.05$ ). For white shrimps, no differences in odor score between all treatments were observed up to 6 days ( $p>0.05$ ) (Figure 41). Similarly, the higher odor score was observed in whole shrimp than did the decapitated shrimp ( $p>0.05$ ). All samples were not acceptable after storage for 10 to 12 days. The unacceptability of all samples might be caused by the strong ammoniacal odor. This was concomitant with the higher TBARS values of both species kept in ice for an extended time (Figure 28A and B). The fishy and ammoniacal odor could be attributed to the reaction between TMA and fish oil (Yamakata and Low, 1995). Lipid oxidation is a major cause of deterioration in meat quality (Gomaa *et al.*, 1996). It limits the storage or shelf-life of meat exposed to oxygen under conditions, where microbial spoilage is prevented or reduced such as refrigeration or freezing. The products of fatty acid oxidation produce off-flavors and odors usually described as rancid (Gray and Pearson, 1994).

The changes in odor score of cooked black tiger shrimp and white shrimp were coincidental with those of raw samples (Figure 41). Generally, odor score of cooked black tiger shrimp and white shrimp increased with increasing storage time. Shrimp odor became stronger when heated. Shrimp aroma is weakly smell before cooking but the strong pleasant arises after heating (Morita *et al.*, 2001). Seafood-like odors of unsaturated methyl ketones among more than 100 compounds including 40 sulfur and/or nitrogen containing compounds in cooked shrimp volatiles were found (Kubota and Kobayashi, 1988). Those compounds might be lost during the extended storage, resulting in the lower acceptability in odor as indicated by the higher score.

Flavor scores of cooked black tiger shrimp and white shrimp during ice storage are shown in Figure 42. Flavor scores of cooked samples of both species increased as storage time increased regardless of decapitation and icing method ( $p < 0.05$ ). No significant differences in flavor score between treatments of both species were observed throughout the storage. The higher flavor score was found in whole shrimps ( $p < 0.05$ ). According to the criterion for acceptability limit, score greater than 5 indicates unacceptability. Thus, shrimps could be kept in ice up to 8 days to obtain the acceptability. Final temperature was important to the sensory profile. (Johanson *et al.*, 1992).

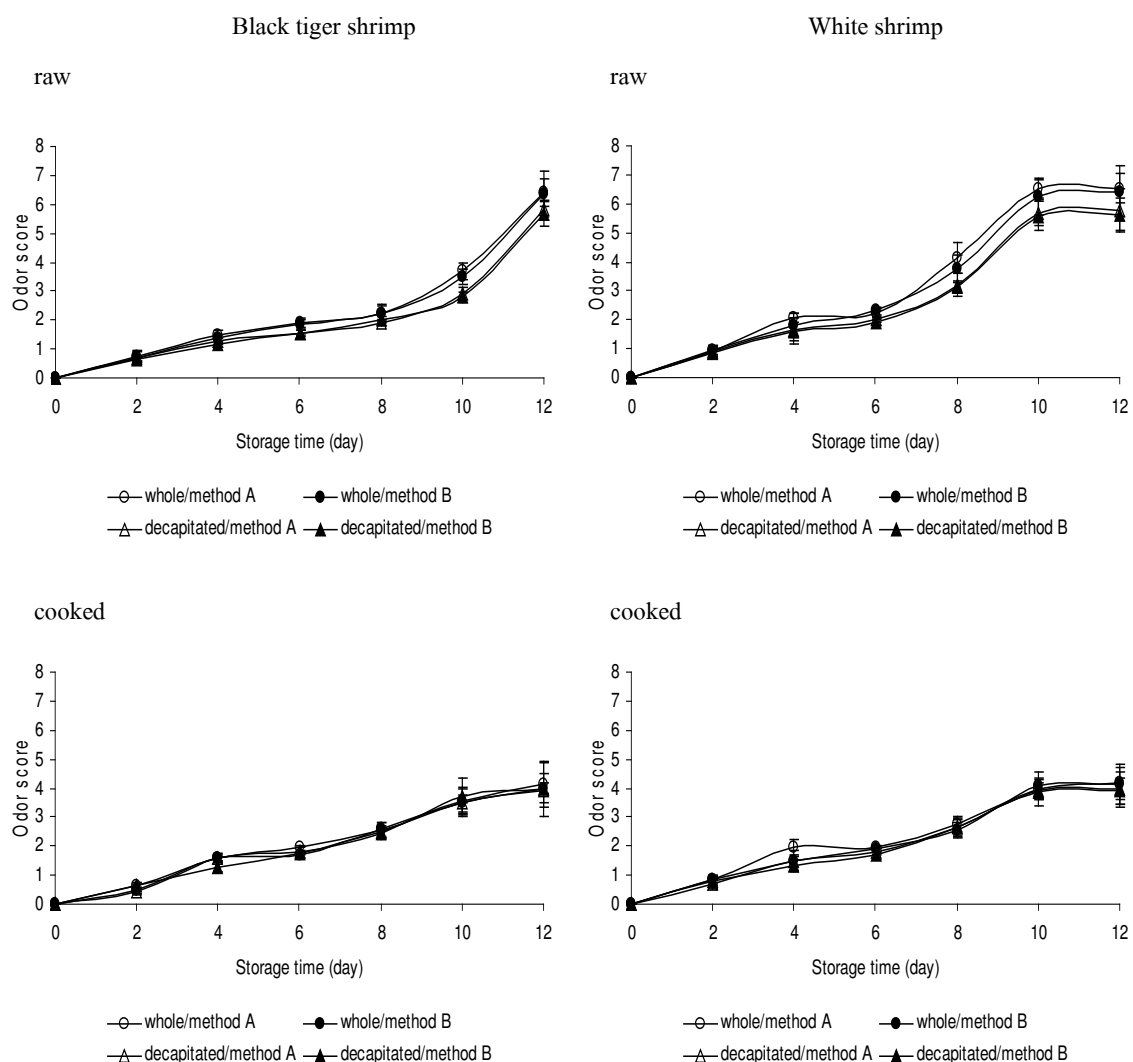


Figure 41 Changes in odor score of raw and cooked whole and decapitated black tiger shrimp and white shrimp with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations. Score 0 = Strongest Pass; Score 10 = Strongest Fail.

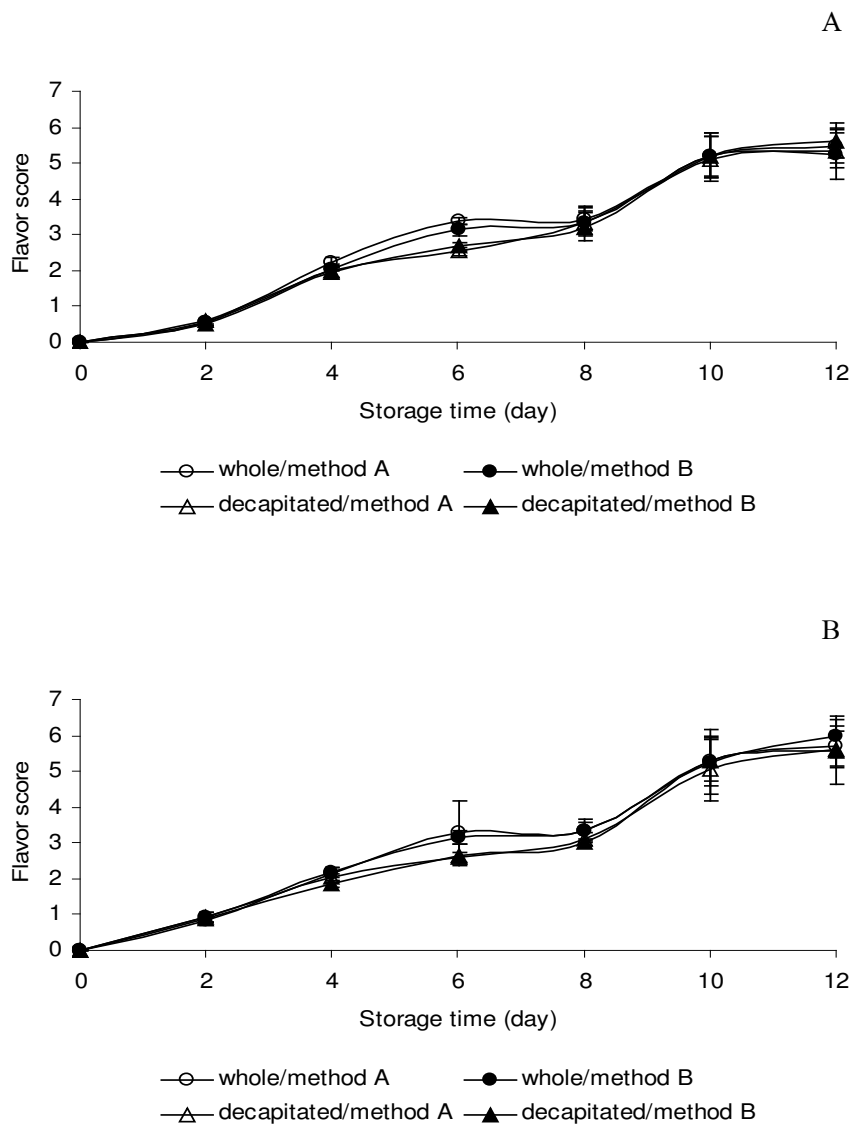


Figure 42 Changes in flavor score of cooked whole and decapitated black tiger shrimp (A) and white shrimp (B) with different icing methods during the storage. Bars represent the standard deviation from triplicate determinations. Score 0 = Strongest Pass; Score 10 = Strongest Fail.

The increases in total quality, appearance and odor scores were concomitant with the increases in TVC (Figure 37). Therefore, it was most likely that the lowered acceptability of both shrimps mainly associated with spoilage bacteria, which contributed to the softening or production of offensive odor and flavor. So, keeping the shrimps in ice for the short time could be a promising means to retard the spoilage and to maintain the quality of both shrimps.