CHAPTER 1

INTRODUCTION

Seafood and seafood products have attracted considerable attention as an important source of nutritional human diet. Apart from their delicacy, crustacean species such as shrimp, crab, lobster, etc., consist of amino acids, peptides, protein and other useful nutrients. Penaeid shrimps have become the economically important species for Thailand and are widely cultured in ponds. In Thailand, approximately 50 species of Penaeus are found (Chaitiamvong and Supongpan, 1992). Among those, black tiger shrimp (*Penaeus monodon*) and white shrimp (*Penaeus vannamei*) are commonly cultured and exported with a catch volume over 1,000 tons per year and Thailand exported 249,570 tons of shrimp and shrimp products with the value of 2.19 billions US dollars in year 2001 (Suphamongkhon, 2002).

Shrimp meat is an excellent source of protein (Yanar and Celik, 2006). Additionally, shrimp muscle comprises highly unsaturated fatty acid (HUFA) such as eicosapentaenoic (20:5n3, EPA) and docosahexaenoic (22:6n3, DHA) acids considered as the essential fatty acids. (Feliz *et al.,* 2002). Shrimp meat is also a good source of minerals such as calcium (Yanar and Celik, 2006). Nevertheless, the compositions can vary with feed and the environment where they live. Feed quality, stocking density and water quality are the main factors affecting productivity for semi-intensive and intensive culture of Penaeid shrimp (Cruz *et al.,* 1993). Formulated feed plays an important role as the source of nutrients and the protein content in commercial shrimp feeds vary between 30 and 50% (Martinez *et al.,* 2003). Additionally, proximate compositions (Karakoltsidis *et al.,* 1995), fatty acid profiles and cholesterol contents (Luzia *et al.,* 2003) and total carotenoid contents (Yanar *et al.,* 2004) of shrimps were changed seasonally. Both black tiger shrimp and white shrimp have been accepted among consumers differently, possibly owing to the varying compositions and properties.

After death, fish undergoes deterioration via microbial and chemical reactions. Protein is one of major constituents, which is prone to deterioration and changes. Fish proteins can be degraded by endogenous and microbial proteases. The autolysis of nucleotides as well as nitrogenous compounds become more intense after the prolonged storage, particularly with

inappropriate condition (Gill, 1992). The formation of biogenic amines used as the quality index generally occurs (Mieltz and Karmas, 1977). Also, lipid oxidation causing the rancidity takes place (Jadhav *et al*., 1996). To prevent such a deterioration, freezing technology has been successfully applied so far. Despite microbial spoilage can be terminated effectively, quality deteriorations, especially in texture, flavor and color, still take place during frozen storage (Benjakul *et al.,* 2003). Freeze-thaw process can also promote protein denaturation and lipid oxidation, which may affect the texture of fish muscle (Benjakul and Bauer, 2000; Srinivasan *et al.,* 1997). Deterioration of muscle proteins during frozen storage depends on many factors including species, storage temperature, time and enzymatic degradation (Ang and Hultin, 1989; Badii and Howell, 2001; Hsieh and Regenstein, 1989). Those changes influenced by the postmortem storage and freeze-thawing can be varied with species. The information gained regarding the composition and properties of both shrimps as well as quality changes during iced storage or freeze-thawing process can be used to monitor quality, particularly to retard the losses in quality of both shrimps. As a consequence, the prime quality with high market value can be maintained.

Literature Review

1. Chemical composition of fish and shellfish

The main constituents of fresh fish are water (65-80%), protein (15-24%), fat (0.1-22%), carbohydrate (1-3%) and inorganic substances (0.8-2%) (Suzuki, 1981). The relative amounts of these components are generally within the range found in mammals (Mackie, 1994). Protein is a major composition of fish muscle ranging from 15 to 20% (wet weight), but protein content is reduced in spawning period (Almas, 1981). Protein compositions of fish vary, depending upon muscle type, feeding period and spawning, etc. (Suzuki, 1981).

Shrimp meat consists of high protein content (50% dw.) (Barelay *et al*., 1983). However, chemical compositions of shrimps were reported to change seasonally (Yanar *et al.,* 2004). Karakoltsidis *et al.* (1995) reported that the changes in chemical composition of shrimp (*Aristeus antennatus*) and Norway lobster were influenced by seasons (Table 1).

Table 1 Chemical composition (%) of lobster and shrimp at different season

Species	Season	Protein	Fat	Moisture	Ash	Carbohydrate
Lobster (Norway)	Spring	19 ± 0.9	0.2 ± 0.1	78 ± 1.9	2.0 ± 0.1	0.8 ± 0.1
	Fall	17 ± 0.8	0.5 ± 0.1	80 ± 0.6	2.0 ± 0.2	0.5 ± 0.2
	Winter	17 ± 0.9	0.2 ± 0.1	79 ± 0.5	1.0 ± 0.2	3.0 ± 0.3
Shrimp	Spring	19 ± 1.3	0.2 ± 0.5	77 ± 0.3	2.0 ± 0.1	2.0 ± 0.4
	Fall	16 ± 1.4	0.4 ± 0.1	80± 0.4	2.0 ± 0.2	2.0 ± 0.2
	Winter	19 ± 0.2	0.6 ± 0.07	79 ± 0.4	1.0 ± 0.1	1.0 ± 0.6

Source: Karakoltsidis *et al.* (1995)

1.1 Proteins and nitrogenous compounds of fish and shellfish muscle

1.1.1 Proteins

There are different proteins in fish muscle. These proteins perform different tasks and have varying properties (Sikorski *et al.,* 1990). The proteins can be classified into three groups based on solubility as follows:

1.1.1.1 Sarcoplasmic proteins

The sarcoplasmic proteins usually refer to the proteins of the sarcoplasm as well as the components of the extracellular fluid and the sarcoplasm. The sarcoplasmic proteins comprise about 20-35% of the total muscle proteins and are commonly called myogens (Mackie, 1994; Pearsons and Young, 1989). Despite their diversity, sarcoplasmic proteins share many common physicochemical properties. Most are of relatively low molecular weight, high isoelectric pH, and have globular or rod-shaped structures. The sarcoplasmic proteins are extracted by homogenizing the muscle tissue with water or solutions of neutral salts of ionic strength below 0.15. Among the sarcoplasmic enzymes influencing the quality of fish, the enzymes of the glycolytic pathway and the hydrolytic enzymes of the lysosomes are found to be important (Sikorski *et al.,* 1990).

1.1.1.2 Myofibrillar proteins

These proteins can be extracted from the muscle tissue with neutral salt solutions of ionic strength above 0.15, usually ranging from 0.30 to 0.70. The myofibrillar proteins are related with the water holding capacity and the other functional properties of proteins such as gelation, etc (McCormick, 1994). Contractile proteins which are different in size and location in the muscle are listed in Table 2 (Ashie and Simpson, 1997).

- Myosin and Paramyosin

Myosin makes up 50 to 58% of the myofibrillar fraction (Sikorski *et al.,* 1990). About one-third of the total protein in muscle is myosin, the predominant myofibrillar protein of the thick filament. Native molecular weight of myosin is about 500,000 dalton. Myosin consists of six polypeptide subunits, two large heavy chains and four light chains arranged into an asymmetrical molecule with two pear-shaped globular heads attached to long α-helical rod-like tail (Xiong, 1997) (Figure 1). The long tail of the molecule consists of two polypeptides in a coiled alpha-helix- terminating in two globular heads at one end (McCormick, 1994). Myosin is a protein possessing ATPase activity. The globular head regions of myosin bind and hydrolyze ATP to ADP. The activity reaches its maximum with 3-5 mM Ca^{2+}. This activity is solely due to myosin alone, and thus is not essentially affected by the presence of actin (Ochiai and Chow, 2000). Ca^{2+}-ATPase activity is a good parameter to estimate the quality or the extent of deterioration of protein in muscle food (Matsumoto, 1980; Huidobro and Tejada, 1994). Myosin

ATPase is also largely affected by chemical modification of reactive SH residues (SH_1, SH_2). Modification of SH_2 results in inactivation of Ca^{2+}-ATPase (Ochiai and Chow, 2000).

When myosin is digested by trypsin or chymotrypsin for a short period, myosin is divided into two components, a rapid sediment component called H-meromyosin (HMM), and a slow sediment called L-meromyosin (LMM). When HMM is treated with papain, it is divided into a head and a neck part. A head is called S-1 and the neck part is S-2 (Suzuki, 1981). The myosin head contains the actin binding site, ATP site, alkali light chain site, and DTNB [(5,5-dithiobis)-2-(nitrobenzoic acid)] light chain site. The light chains bind to the alpha-helical regions of the heavy chain. The tail portion of the heavy chain molecule in responsible for its association into thick filaments (Foegeding *et al*., 1996).

Table 2 Contractile proteins in food myosystems

Protein	Relative Abundance (%)	Size (kDa)	Location
Myosin	50-60	470	Thick filaments
Actin	15-30	43-48	Thin filaments
Tropomyosin	5	65-70	Thin filaments
Troponins	5		Thin filaments
Troponin-C		17-18	
Troponin-I		20-24	
Troponin-T		37-40	
C-protein	-	140	Thick filaments
α-Actin	-	180-206	Z-disc
Z-nin	-	300-400	Z-disc
Connective/Titin	5	700-1,000	Gap filaments
Nebulin	5	~ 600	N_2-line

Source: Adapted from Ashie and Simpson (1997)

Paramyosin is one of major muscle proteins found in mollusks (Sikorski, 1994). It consists of high basic amino acid and amide content, such as glutamine (20 to 23.5%), aspartic

acid (12%), argenine (12%) and lysine (9%), but low in proline content. Paramyosin, a rod-shaped alpha-helical chains, consists of 2 subunits, which are 120 nm long with a molecular weight ranging from 95,000 to 125,000 dalton per subunit (Foegeding *et al.,* 1996).

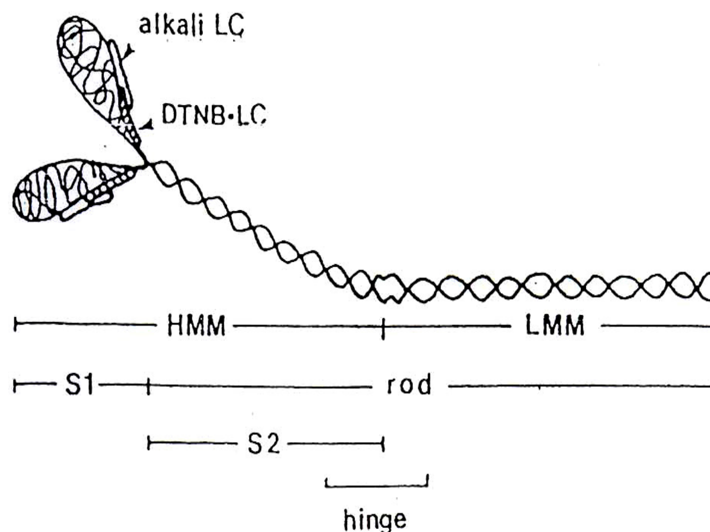

Figure 1 Model of myosin molecule

Source: Xiong (1997)

- Actin

Actin is about 15 to 20% of myofibrillar proten (Sikorski *et al.,* 1990). Acitn is one of three major myofibrillar proteins of thin filaments. Each actin molecule, generally visualized as globular, has a molecular weight of about 40,000 dalton, called G-actin. Polymerized actin molecules via covalent interactions tends to be a helix filamentous molecules, called F-actin. Two F-actins wrap about each other, forming a double helix, called thin filament or I-band, which is associated with tropomyosin and troponin (McCormick, 1994).

- Tropomyosin

Tropomysin, a rod-like molecule, consists of two polypeptide chains, each with a molecular weight range of 34,000-36,000 dalton, which associate to form a coiled helix. Each tropomyosin molecule is about 385 A° long and associates in head-to-tail fashion to form a filament that follows and associates with the coil of the F-actin filament (McCormick, 1994)

(Figure 2a). Tropomyosin is about 5% of myofibrillar protein. Each tropomyosin molecule consists of 7 molecules of G-actins (Foegeding *et al.,* 1996).

- Troponin

Troponin is an asymmetrical protein and consists of three subunits. Troponin T (molecular weight of 37,000 dalton), which is also bound to troponin subunits C and I, links the troponin molecule to the tropomyosin molecule in the I-band. Troponin C (molecular weight of 18,000 dalton) binds Ca^{2+} and confers Ca^{2+} sensitivity to the troponin-tropomyosin-actin complex. Troponin I (molecular weight of 23,000 dalton), the inhibitory subunit, binds tightly to troponin C and actin and only slightly to tropomyosin or troponin T (McCormick, 1994) (Figure 1b).

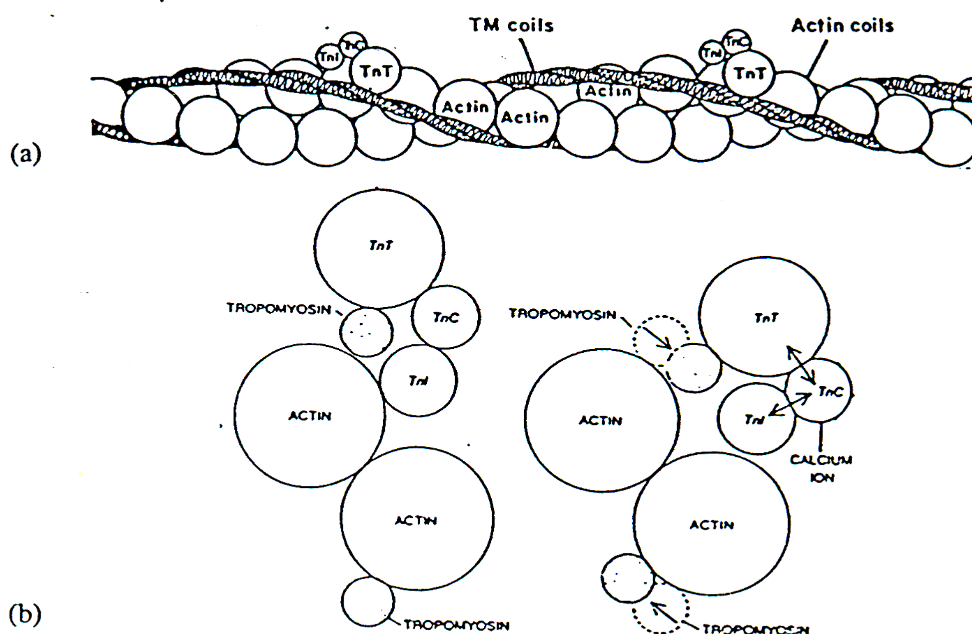

Figure 2 (a) A thin filament of muscle formed by the filament of tropomyosin molecules wound in each of the two grooves of the actin helix. (b) Proposed model for configuration of actin, tropomysin and troponin (Tn) subunits.

Tn T = troponin-tropomyosin subunit

Tn I = troponin-inhibitory subunit

Tn C = troponin-calcium-binding subunit

Source: McCormick (1994)

1.1.1.3 Stromal proteins

The stroma is composed of the main connective tissue proteins, such as collagen and elastin. The stroma is the residue after extraction of the sarcoplasmic and myofibrillar proteins. Generally, the stroma is insoluble in dilute solutions of hydrochloric acid or sodium hydroxide (Sikorski *et al.,* 1990).

Collagens in the muscle of marine animals play a key role in maintenance of meat texture. The musculature of several crustaceans has been shown to contain collagenous proteins (Yoshinaka *et al.,* 1989). The amino acid composition of crustacean collagens is richer in several essential amino acids making the biological value of such collagens significantly higher than bovine and other mammalian muscle collagens (Sikorski *et al.,* 1984 cited by Ashie and Simpson, 1997). A collagen molecule is a helical alpha chain. Each alpha chain twists into a left-handed polyproline helix with three residues per turn. The helical alpha chains contain a GLY-X-Y sequence repeated 340 + 2 times per molecule, where X or Y are often proline or hydroxyproline. The three helical alpha chains are wound into a right-handed superhelix, which forms a molecule about 1.4 nm wide and 300 nm long. Fibrillar collagens are, thus, about one-third glycine and one-quarter proline and hydroxyproline with a molecular weight of about 300,000 dalton (McCormick, 1994). Collagen content in animal tissue changes, depending upon the types of animals, animals age and maturity. The steady increase in mature collagen cross-linking is due to progressive and ongoing cross-linking reactions that occur within fibrillar collagen and with the slowing of collagen synthesis rates as animals reach maturity (McCormick, 1994).

At least 14 different collagen types have been identified. However, the major ones are types II and III. Type I collagen is predominant in the epimysial membrane, type II and III in the perimysium, and type III, IV and V in the muscle endomysium (Hultin, 1985). Sivakumar *et al.* (2000) proposed that type V like collagen were widely distributed in marine invertebrates, particularly crustaceans and molluses. Yoshinaka et al. (1990) purified a type V like collagen from muscle of kuruma prawn, *Penaeus japonicus* by pH fractionation and phosphocellulose chromatography. At least three types of collagen was found in the muscle of kuruma prawn. Two of them, called AR-I and AR-II collagens, were isolated from the pepsin-solubilized collagen (Figue 3) (Yoshinaka and Mizuta, 1999). The subunit composition of AR-I

collagen, a major component, was proved to be $(\alpha\ I)_3$ homotrimer. Its amino acid composition was similar to that of Type V collagen rather than Type I collagen. AR-II collagen, a minor molecular component, was found to have disulfide bonds in the molecule. Its amino acid composition was similar to that of AR-I collagen. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis pattern of AR-II collagen suggested the complexes of its structure. The major collagen in shrimp (*Penaeus indicus*) was found to be a homotrimer of α 1 chain, similar to type V collagen of vertebreates (Sivakumar *et al.,* 1997).

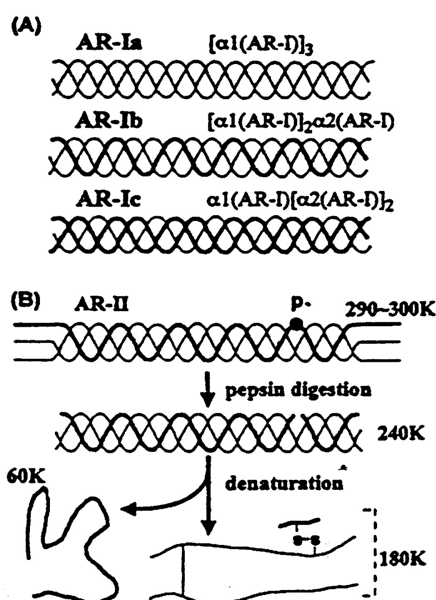

Figure 3 Collagen structures of shrimp

A = subunit composition of type AR-I collagen

B = proposed structure of type AR-II collagen

C = pepsin-sensitive region

Source: Yoshinaka and Mtizuta (1999)

The musculature of marine crustaceans like prawn has a flexible musculature and collagens with more crosslink and higher denaturation temperature than fish (Sivakumar *et al.,* 2000). However, Crawford (1981) reported that shrimp collagen was more susceptible to hydrolysis by proteinase than fish collagen.

1.1.2 Non-protein nitrogenous (NPN) compounds of fish and shellfish muscle

The content of NPN compounds in fish depends primarily on the species, state of freshness, habitat and other effects (Ikeda, 1979). Those compounds account for up to 55% of the total nitrogen in shark muscle tissues. Approximately 85% of NPN compounds in muscle tissues of marine fish and shellfish is composed of free amino acids, amines, amine oxides, guanidines, nucleotides and their breakdown products, urea and quaternary ammonium salts (Ikeda, 1979). The contribution of NPN compounds to flavor of seafoods is of paramount importance (Table 3) (Fine, 1992).

Table 3 Distribution (%) of non-protein nitrogenous (NPN) compounds in some seafoods

Species	Free amino acids	Peptides	Nucleotides	Creatine/creatinine	betaines	Others
Mackerel	25	5	10	35	-	25
Shark	5	5	5	10	-	75
Shrimp	65	5	5	-	5	20
Crab	75	5	15	-	5	-
Squid	50	5	15	-	10	20
Clam	35	5	35	-	15	10

Source: Adapted from Fine (1992)

1.2 Lipids and fatty acids in fish and shellfish muscle

Lipids are one of major components affecting the quality of fish and shellfish. Lipid contents vary with fish species. Total lipid in shellfish including mollusks and crustacean is usually 1 to 2% (King *et al.,* 1990). Edible portions of marine shrimp consist of 1-2% of lipid (Johnston *et al*., 1988). Chanmugam *et al.* (1983) reported that fresh water shrimp muscle (*Machrobrachium resenbregii*) comprised about 3.8% of lipid. Lipid compositions of shrimp include triglyceride, glycolipid and phospholipids (Table 4). Among all lipids, phospholipids are the predominant lipid class in crustacean (Yepiz-Plascencia *et al.,* 2000).

Table 4 Lipid compositions of shrimp meats (mg/100 g meat)

Class	tissue (mg/100 g)	%
neutral lipids	430.7 ± 20.1	36.0 ± 1.7
glycolipid	22.8 ± 2.9	1.9 ± 0.3
phospholipids	742.0 ± 40.7	62.1 ± 3.4
total	1195.5 ± 63.3	

Source: Johnston *et al.* (1988)

Lipids are a major source of energy in marine invertebrates, including shrimp. Furthermore, they are involved in several essential processes for their growth, molting and reproduction (Yepiz-Plascencia *et al.,* 2000). Lipid compositions of fish depend on many factors including sex, growth stage and season (Bottino *et al*., 1980). Crustaceans require dietary lipid as a source of essential fatty acids (EFA) and other lipid classes such as phospholipids (PL) and sterols. In term of lipid nutrition, total PL content recommended by Akiyama *et al.* (1992) is of 2% of diet. Gong *et al.* (2000) observed a significant interaction between dietary PL and cholesterol on growth of juvenile *L. vannamei.*

Fatty acids in crustacean includes 16:0, 16:1ω7, 18:1ω9, 20:5ω3 and 22:6ω3, and consist of 55-90% of total fatty acids (Karakoltsidis *et al*., 1995). Four fatty acids are essential for *Penaeus monodon*: linoleic (18:2n-6, LOA), linolenic (18:3n-3, LNA), eicosapentaenoic (20:5n-3, EPA), and docosahexaenoic (22:6n-3, DHA) acids with latter two n-3 highly unsaturated fatty acids (HUFA) being the most indispensable (Catacutan, 1991; Merican and Shim, 1996; Glencross and Smith, 2001; Glencross *et al.,* 2002). Johnston *et al.* (1988) reported that ω6 was the major fatty acids in fresh water shrimp, while ω3 was dominant in marine shrimp. Fatty acid profiles and cholesterol contents of shrimps were reported to change seasonally (Luzia *et al.,* 2003). Although the fatty acid composition of PL appears to be critical for their nutritional functionality in crustaceans, few studies have compared the nutritional effects of PL and fatty acid with different origins (Kanazawa *et al.,* 1979c, 1985; Coutteau *et al.,* 2000).

Due to the presence of highly unsaturated fatty acids, the oxidation of fish muscle is higher than poultry, pork and lamb, respectively (Hsieh and Kinsella, 1989). Lipid oxidation is of great concern to the food industry and consumers since it contributes to the development of

poorer flavor, color and texture, reduces nutritive value and produces potentially toxic reaction products (Namiki, 1990).

1.3 Carotenoid

Body color of crustaceans is the one of the important factors that affects their commercial value. Astaxanthin is a red-orange carotenoid found in fish and shellfish. It is mainly associated with the color of invertebrate animals such as shrimp, crabs, and lobsters. Generally, it presents in crustaceans as a protein-pigment complex (Armenta-Lopez *et al.,* 2002). Astaxanthin is the predominant carotenoid in penaeids (Ishikawa *et al.,* 1966; Katayama *et al.,* 1971, 1972; Okada *et al.,* 1994). Three forms of astaxanthin, namely diester, monoester, and free, were found in black tiger shrimp (Okada *et al.,* 1994). The main carotenoid in the carotenopritein was free astaxanthin, and no significant difference was found between astaxanthin diester and astaxanthin monoester (Okada *et al.,* 1995). This complex can be green, purple, or blue in the living animal, acquiring a red color when subjected to heat treatment (Britton, 1996). Carotenoids as well as carotenoproteins are responsible for the color of crustaceans. When cooked, the body color of prawns becomes red and the depth of the color depends upon the carotenoid content (Okada *et al.,* 1994).

Natural carotenoids play an important role in fish reproduction (Chen and Meyers, 1983). Since crustaceans are unable to synthesize carotenoids, astaxanthin or appropriate precursors must be supplied in the diet (Meyers and Latscha, 1997). Dietary supplementation of astaxanthin or its precursors improved (Chien and Jeng, 1992; Liao *et al.,* 1993) or corrected (Mensaveta *et al.,* 1993) the color of penaeids, especially those intensively cultured, for better market price (Liao and Chien, 1994). Yanar *et al*. (2004) reported that caroteinoid contents of shrimps (*Penaeus semisulcatus* and *Metapenaeus monoceros*) varied with seasons.

In nature, protein-pigment interaction increases carotenoid stability (Britton 1996). The use of proteolytic enzymes to disrupt the protein-pigment bond, increased carotenoid extraction by 58% (Cano-López *et al.,* 1987) or spitted the chitin-pigment interaction, resulting in protein-enriched pigments (Hansen and Llanes, 1994). Protein-free astaxanthin can be used in a variety of products such as foods, feeds, cosmetics, and pharmaceuticals. The red-orange color of astaxanthin is associated with high quality in some foods such as salmon and trout; therefore, the inclusion of this pigment in their diets results in improved quality.

1.4 Mineral content

Invertebrates are rich in minerals (Yanar *et al.,* 2006). Major sources of minerals to marine organisms are sea water and feed (Ichihashi *et al.,* 2001). Yanar *et al.* (2006) reported that Ca, K, P, Na and Fe were observed as the main minerals in shrimp muscle (*Penaeus semisulcatus* and *Metapenaeus monoceros*). Calcium is essential for hard tissue structure, blood clotting, muscle contraction, nerve transmission, osmoregulation and as a cofactor for enzymatic procession (Lovell, 1989). Most of aquatic species can absorb Ca directly from the surrounding condition to meet their Ca requirement (Davis and Gatlin, 1996). Lall (1989) reviewed that dietary Ca requirement was affected by the water chemistry, species and dietary P level. However, *L. vannamei* and *Penaeus monodon* in seawater and *Metapenaeus macleayi* in brackish water had no dietary Ca requirement and excessive dietary Ca could depress shrimp growth (Penaflorida, 1999). Among the minerals required by penaeid shrimp, P is crucial because of its limited availability under rearing conditions. P is directly involved in all energy-yielding reactions and has an integral role in cellular functions, as it is a key component of nucleic acids, phospholipids, phosphoproteins, ATP and several key enzymes (Lovell, 1989). However, mineral contents of the two species of shrimp (*Penaeus semisulcatus* and *Metapenaeus monoceros*) varied with season, except for the Ca content in *Penaeus semisulcatus* (Yanar *et al.,* 2006).

Transition metals are also found in the pigments of fish and shellfish. Irons in heme proteins are the major prooxidants in muscle foods (Decker and Hultin, 1992). Cu ion is found in hemocyanin, a pigment in blood of crustacean (Decker and Tuczek, 2000).

1.5 Nucleotide

Nucleotide in fish muscle is the derivative of purine from the catabolism of adenosine triphosphate (ATP). The degradation sequence of ATP in the fish species starts from ATP to adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), inosine (Ino) and hypoxanthine (Hx), respectively (Figure 4) (Gill, 1992). Shrimp (*Penaeus monodon*) contains the highest total purine content of any commercial seafood (Lou *et al.,* 1996). According to Konosu and Yamaguchi (1982), the adenosine triphosphate (ATP) and AMP are the dominant nucleotides in crustacean and mollusks, immediately after catching. Mendes *et al.* (2001) reported that AMP is the major nucleotide in red shrimp caught off Algarve (Portugal), with concentrations of 12 to 14 μmol/g, whereas IMP content was less than 2 μmol/g. In Japanese

prawn (*Pandalus hypsinotus*), Arai (1966) proposed two pathways for the degradation of ATP. One involved the deamination of AMP to IMP, and the second involved dephosphorylation of AMP to adenosine, followed by deamination to Ino. Stone (1970) and Fatima *et al.* (1981) considered the major pathway for adenine nucleotide degradation to be deamination of AMP to IMP in several shrimp species. Matsumoto and Yamanaka (1991) found IMP accumulated in kuruma prawn during storage. IMP play an important role in taste of fish and shrimp (Foegeding *et al.,* 1996). Raw shrimps have a higher purine content than cooked shrimp (Lou, 1997a). Quality and quantity of purine compounds in food might change during storage and processing (Lou *et al.,* 1997). After death, fish muscle produces ATP, the major source of readily available contractile energy, in the absence of oxygen. In the meantime, ATP levels are quickly depleted as the muscle enters the rigor process (Figure 4) (Gill, 1992). However, Huidobro *et al.* (2001) showed no differences in nucleotide derivatives in gilthead sea bream arising from slaughter by immersion in iced water, asphyxiation in air, concussion, or rested harvesting.

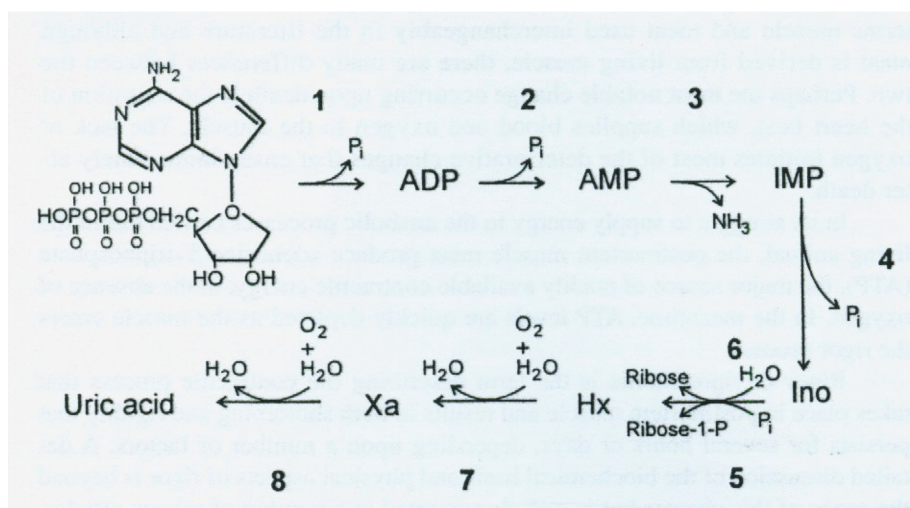

Figure 4 Postmortem ATP degradation in fish. Enzymes include: (1) ATPase; (2) Myokinase; (3) AMP deaminase; (4) 5′nucleotide; (5) Nucleoside phosphorylase; (6) Inosine nucleosidase; 7, 8. Xanthine oxidase. ADP, adenosine diphosphate; AMP, adenosine monophosphate; IMP, inosine monophosphate; Ino, inosine; Hx, hypoxanthine; Xa, xanthine, Pi, inorganic phosphate.

Source: Gill (1992)

Rigor or rigor mortis is the term describing the contractile process that takes place in postmortem muscle and results in both shortening and rigidity. Several factors influencing the onset and strength of *rigor mortis* in fish include species, temperature, pre-slaughter handling and killing method (Hutin, 1984). Rigor mortis of bartailed flathead started after 10 h at 0°C and 13 h at 10°C (Iwamoto *et al,* 1990). Handling and crowding affected rigor mortis contraction, muscle pH, and fillet texture of Atlantic salmon (*Salmo Salar*) (Gómez-Guillén *et al.,* 2000). Rigor contraction was therefore closely correlated to fish quality (Veiseth *et al.,* 2006).

1.6 DSC thermogram of fish muscle

The transition due to denaturation is often referred to in term of its peak maximum temperature (T_{max}). T_{max} of the first peak of muscle proteins is assumed to correspond to myosin denaturation. Peak 2 is stable transition observed in the thermograms and is assumed to correspond to actin denaturation. T_{max} of myosin was different among fish species. The highest T_{max} for all species (e.g. sardine, stone flounder, walleye Pollack, sea bream, carp, greenling, bigeye tuna and yellow tail), except rainbow trout was approximately as high as T_{max} (46°C) for rabbit (Ogawa *et al.,* 1993). Thermal denaturation of the myosin was dependent on pH and ionic strength (Wright and Wilding, 1984). Davies *et al*. (1988) found that snapper myosin showed the distinct peak with relatively high T_{max}. As the pH was increased, the transition peak became distinguishably less stable, as shown with a sharp decrease in T_{max}. Whole cod muscle showed two maximal transitions on DSC thermogram with T_{max} about 45 and 75°C (Hasting *et al.,* 1985) and whole muscle of fresh hake also showed two endothermic transitions with T_{max} values of 46 and 75°C (Beas *et al.,* 1990). DSC thermogram revealed one peak at 49°C for collagen extracted from shrimp (*Penaeus vannamei*) and another at 65°C for type I collagen from bovine Achilles tendon (Brauer *et al.,* 2003). Differential scanning colorimetric (DSC) study revealed three transitions corresponding to the thermal denaturation of myosin and paramyosin, connective tissues and actin of cuttlefish (*Sepia pharaonis*) muscle, at temperatures of 49.8–50.3, 59.8–60.3 and 74.7–78.8 1°C, respectively (Thanonkaew *et al.,* 2006). The difference in T_{max} of the transitions among the fish species seems to be correlated with the habitat temperature of the fish (Davies *et al.,* 1988).

2. Post mortem changes of fish

2.1 Changes of proteins

Post mortem tenderization is one of the most unfavorable quality changes in fish muscle. A proteolytic degradation of myofibrillar and connective tissue components is observed during the extended storage. Myofibrillar proteins fraction in muscle of Monterey sardine was unstable during ice storage (Pacheco-Aguilar *et al.*, 2000). The participation of various proteinases in autolytic processes of ice-stored fish depends on the following: location of the enzymes in cytosol and/or factors affecting tissue compartmentization, presence of activators or inhibitors and the susceptibility of the proteins responsible for muscle integrity to in situ cleavage by the respective enzymes (Ladrat *et al.,* 2003). The decrease in the relative amount of myosin heavy chain (MHC) and a concomitant increase in the number and intensity of bands of molecular size about 100 kDa cross-reacting with anti-MHC antiserum were found in *Penaeus borealis* and other prominent features were the disappearance of bands of about 67 and 50 kDa after 24 h and the appearance of a band of slightly less than 50 kDa after 5 h of iced storage (Martinez *et al*., 2001). Eckhoff *et al*. (1998) reported that insoluble collagen in salmon (*Salmo salar,* L) decreased gradually during 15 days of the storage in ice. The relationship between collagen content and texture was further confirmed by Hatae *et al.* (1986), who showed that a high collagen content resulted in a firm meat. Raw fish meat softened rapidly during chilled storage and histological examinations were shown to be caused by disintegration of collagen (Sato *et al.,* 1991). The initial steps in deterioration of raw fish during its storage on ice consist of hydrolytic reactions catalyzed by endogenous enzymes, which produce nutrients that allow bacteria proliferation (Busconi *et al.,* 1989). A change in microstructure of *Macrobrachium rosenbergii* during storage in ice was observed (Nip and Moy, 1988). Rowland *et al.* (1982) reported that substantial morphological changes due to proteolysis in the tails of *M. rosenbergii*.

2.2 Changes of biogenic amines

Decomposition in seafood encompasses several aspects of product deterioration including enzymatic spoilage caused by microorganisms, autolytic enzymatic breakdown, and rancidity (Benner *et al.,* 2003). Biogenic amine formation in seafood is important since histamine, and possibly other biogenic amines are responsible for scombrotoxic fish poisoning (Taylor, 1986). Furthermore, biogenic amines have been used as chemical indicators of seafood quality (Mieltz and Karmas, 1977). Fish muscle is capable of supporting the bacterial formation of a wide variety of amine compounds via the decarboxylation of amino acids. Biogenic amine formation in fish and fish products depends on the amino acid content of fish, the presence of bacterial biogenic amine decarboxylases and favourable environmental conditions (Silla-Santos, 1996). 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, areginine, tyrosine, trytophan and phenylalanine, respectively (Figure 5) (Ozogul *et al.,* 2006). Biogenic amines are non-volatile compounds, which are formed at very low levels in fresh fish, and their accumulation is related to bacterial spoilage (Fernandez-Salguero and Mackie, 1987). Putrescine was the main biogenic amine formed in sea bass (*Dicentrarchus labrax*) during iced storage (Paleologos *et al.,* 2004). In shrimp, putrescine has been suggested as an index of decomposition (Mietz and Karmas, 1978). Bacteria belonging to the family Enterobacteriaceae had the ability to produce both cadaverine and putrescine during storage in ice of fish (*Lethrinus miniatus*) and shrimp (*Penaeus semisulcatus*) (Lakshmanan *et al.,* 2002). Putrescine in shrimps (*Litopenaeus setiferus, L. brasiliensis* and *L. vannamei*) at a reject level of 3 ppm, confirmed sensory evidence of decomposition more frequently than did cadaverine or indole at reject levels of 3 ppm or 25 μg/100 g, respectively (Benner *et al.* 2003). A good correlation was found between sensory evaluation and levels of putrescine and cadaverine in skipjack tuna (Sims *et al.,* 1992). Mietz and Karmas (1997) proposed the biogenic amine index (BAI) to evaluate the quality of rockfish, salmon, lobster and shrimp.

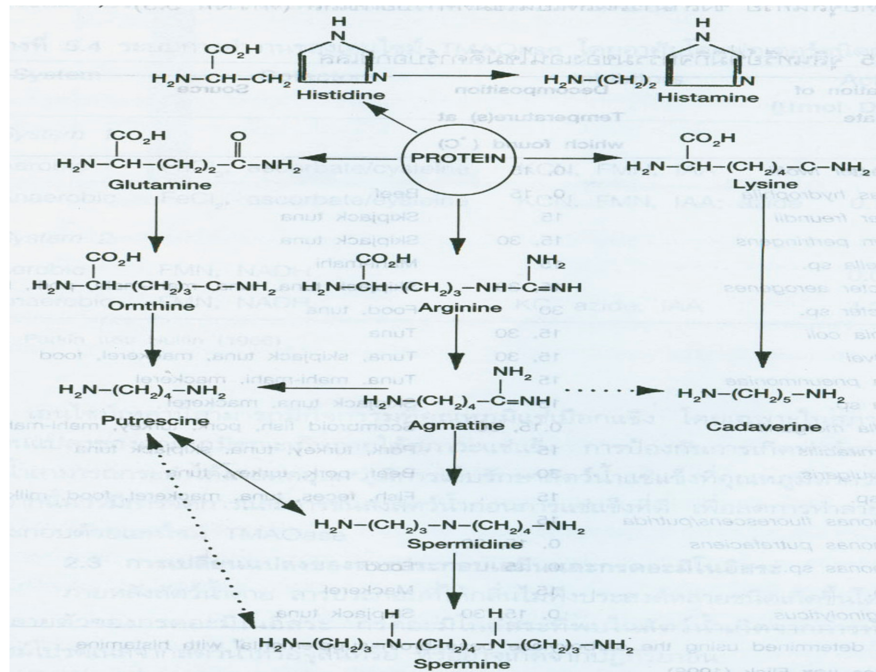

Figure 5 Formation of biogenic amines

Source: Rawles and Flick (1996)

2.3 Changes of lipids

Changes in lipid components of postmortem fish and shellfish are lipolysis and oxidation of lipids. The hydrolysis of lipid was catalyzed by lipases and phospholipases and free fatty acids were produced during postmortem storage. Lipid oxidation also occurs during postmortem storage (Foegeding *et al.,* 1996). The changes in fish lipids are directly and indirectly responsible for the quality deterioration of fish. Lipid oxidation and interaction of its products with nonlipid components such as protein occurred (Pacheco-Aguilar *et al.,* 2000). Postmortem hydrolysis of triglyceride or phospholipids and oxidation of lipid especially, unsaturated fatty acids (PUFAs), were dependent on fish/ice ratio and storage temperature. Chanmugam *et al.* (1983) reported the increase in PUFAs in phospholipids but no changes in PUFAs of total lipid of fresh water shrimp during stored in ice after 7 days. However, no changes in fatty acids in brown shrimp were observed after stored in ice for 18 days (Bottino *et al.,* 1979). The autoxidation of major fatty acids of meat follows the order C18:0 < C18:1 < C18:2 < C18:3 (Shahidi, 1994a). Since seafoods contain highly unsaturated fatty acids, the oxidation of muscle from fish is higher than poultry,

pork and lamb, respectively (Tichivangana and Morrissey, 1985). Aubourg *et al.* (2004) reported that peroxide formation in chilled turbot was very slow during chilled storage. After 29 days of iced storage, the concentration of peroxides was below 4.00 meq kg^{-1} lipids. The lipid oxidation occurred at a lower rate in turbot than in other fish species (Jessen, 1996).

Pacheco-Aguilar *et al.* (2000) reported an increase in TBA values of Monterey sardine stored in ice from day 2 to day 11, which was 4.3 mg MA/kg muscle to 37.23 mg MA/kg muscle. TBA index is the most used indicator for advanced lipid oxidation (Nishimoto *et al.,* 1985). St. Angelo (1996) reported that iron bound to protein such as myoglobin, heamoglobin and ferritin may be released during postharvest storage and cooking, resulting in the initiation of lipid oxidation.

2.4 changes in nucleotide

Nucleotides and their breakdown products have been used as the index for the evaluation of crustacean quality (Mendes *et al.,* 2002). The decrease in ATP (Adenosine triphosphate) and the increase in ADP, AMP, IMP, Ino and Hx during storage of tropical shrimp (*Pandalus borealis*) were reported (Ban *et al.*, 1998). Mendes *et al.* (2001) reported that nucleotide in Norway lobster and red shrimp from the South of Portugal immediately changed after catch and after 72 h of ice storage. Immediately after harvest, within 1 to 2 min after crustaceans landing on deck, AMP was the predominant nucleotide (9.3–11.8 mmol/g) in Norway lobster (*Nephrops norvegicus*) and red shrimp (*Aristeus antennatus*). This accumulation of AMP is due to a highly reduced or nonexistent AMP-deaminase activity (Yokoyama *et al.,* 1992). The K value, which is a ratio of inosine (Ino) and hypoxanthine (Hx) to the sum of ATP, ADP, AMP, IMP, Ino and Hx, has been proposed as a freshness index of seafood (Saito *et al.,* 1959).

Lou (1998) reported that the increase of Hx of black tiger shrimp storage at 22°C ranged from 1.68 to 3.67 (mg/g dw.). The changes in purine content of shrimp during different storage conditions indicate that AMP deaminase, adenosine deaminase or xanthine oxidase may be important in the breakdown pathway of nucleotides in shrimp muscle. However, the accumulation of Ino during ice storage in some other shrimp species supports the adenosine deamination pathway (Cheuk *et al.,* 1979). Thus, it is difficult to determine which nucleotide catabolites may be involved in the K value to effectively estimate the freshness of a given species of shrimp. The K-value of fresh rohu (0.2%) increased linearly with storage time and reached 60% by 25 days of

iced storage (Mohan *et al.,* 2006). Pacheco-Aguilar *et al.* (2000) reported that final K-value was 50.7% after storage in ice for 15 days. Storage temperature has influence on the changes of nucleotide. K value, which is a ratio of Ino and Hx to the sum of ATP, ADP, AMP, IMP, Ino and Hx, has been proposed as a freshness index of seafood (Saito *et al.,* 1959). The K-value increased linearly with storage time and appeared to be a good index of freshness for the two brackish water fish (Lakshmanan *et al.,* 1996). K-value of fishes vary with species, with threadfin bream having K-value of 35% as limit of acceptability (Yongsawtdigul and Park, 2002) while Catla have a acceptability limit when K-value of 55% was obtained. Lakshmanan *et al.* (1996) reported a K-value of 53% for *Labeo rohita* as limit of acceptability, while for *Scophthalmus maximus* a K-value of 75–85% was considered as the limit of acceptability (Ozogul *et al.,* 2005). K-value of 20% is generally regarded as the optimum freshness limit of shrimp, while 60% is considered as the rejection point (Ehira, 1976).

2.5 Changes in melanosis

Enzymatic browning in many food systems generally causes an undesirable appearance. Melanosis results in greatly decreased consumer acceptance and economical losses to the industries. Postmortem discoloration in crustacean species has been linked to the presence of polyphenoloxidase in the hemolymph (Boon *et al.,* 1993). 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 (Figure 6) (Lerner, 1953; Rolle *et al.,* 1991). Polyphenoloxidase (PPO) has been shown to play a central role in the innate immune system of crustaceans (Soderhall and Smith, 1986) and contribute to wound repair and sclerotization of the cuticle post-molt (Soderhall and Cerenius, 1992). PPO catalyzes the hydroxylation of *o*-monohydroxyphenols to *o*-dihydroxyphenols and the subsequent oxidation of *o*-dihydroxyphenols to quinones. The quinones are highly reactive and undergo nonenzymatic oxidation and polymerization to produce melanin pigments (Soderhall and Cerenius, 1992).

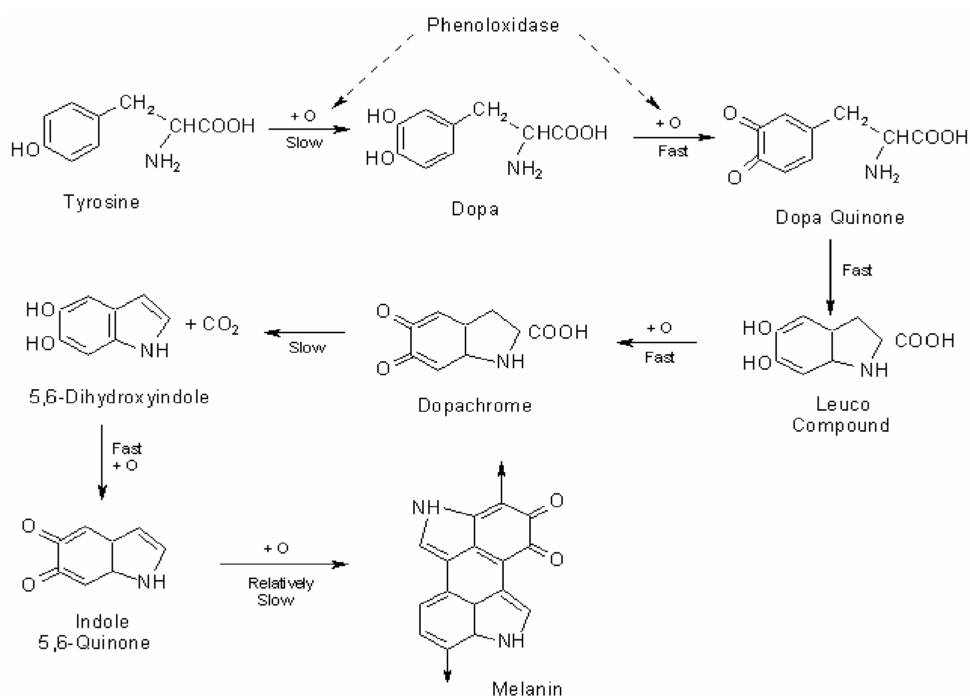

Figure 6 Formation of melanin from tyrosine

Source: Lerner (1953)

Adachi *et al.* (2001) reported that hemocyanin (Hc) from plasma of kuruma prawn was characterized as a potent inducer of black spots during storage. In addition, PO activity of hemocyanin (Hc) in crab, lobster, octopus, and tarantula was reported (Salvato *et al.,* 1998). Hc, as well as PO, is a copper protein found in 2 phyla, mollusks and arthropods. In crustaceans, this oxygen carrier/storage protein is expressed from the hepatopancreas and located at quite high concentrations in plasma, comprising 90 to 95% of the total plasma protein (Van Holde and Miller, 1995), while only a trivial amount of PO exists in hemocytes.

Norway lobsters rapidly develop black spots or melanosis during iced storage. Menlanosis occurs in the integument under the carapace in crustacean (Ogawa *et al*., 1984). Yamagata and Low (1995) found black spot in banana shrimp stored in ice for 2-4 days and the unacceptability caused by black spot were observed at days 6 of storage. Melanosis score was positively correlated with yellowness and inversely with lightness (Martınez-Alvarez *et al.,* 2007).

Although the presence of black spots is not dangerous to human health, it reduces their marketability. Many approaches have been used to prevent the reaction by eliminating the essential components, including oxygen, copper, or substrate needed for polyphenoloxidase (PPO) activity (Kim *et al.,* 2000).

2.6 Changes in microbiology

Shrimp is a very perishable product, and postmortem changes occur rapidly, compared with fish. Microbial contamination was the most important concern in fish processing, especially during refrigerated storage (Gram and Huss, 1996). The high content of free amino acids and other soluble non-nitrogenous substances, which partly contribute to the desirable, delicate sweet taste of shrimp (Konosu and Yamaguchi, 1982), can also serve as easily digestible nutrients for microbial growth. It has been reported by various investigators that the microflora associated with live freshwater and marine crustaceans reflect the microbial population of the waters, in which they live (Liston, 1980). Initial counts of total bacteria count in pink shrimp reached about 2.4–2.7 log cfu/g showing that a correct handling was performed onboard (Huidobro *et al.,* 2002). However, Jeyasekaran et al. (2006) reported that fresh white shrimp carried a bacterial flora of *Aeromonas Pseudomonas, Vibrio, Flavobacterium and Serratia*, especially *Aeromonas* constituting about 38% of the flora. Jeyaweera and Subasinghe (1988) found that *Micrococcus, Aeromonas* and *Moraxella* were dominant in the shrimp, *Penaeus indicus*, stored in ice. *Pseudomonas*, *Aeromonas hydrophila*, *A. veronii boivar* and *Shewanella putrefaciens* were identified as the dominant spoilage organisms of farm reared *M. rosenbergii* stored in ice (Lalitha *et al.,* 2006). Lakshmanan *et al.* (2002) reported that the total mesophilic bacteria in fresh fish (*Lethrinus miniatus*) and shrimp (*Penaeus semisulcatus*) were 10^5cfu/g. Ice storage decreased mesophilic bacteria by one log during the initial period (3-6 days) and later on steadily increased to 10^6cfu/g at the end of storage (12 days) and the total mesophilic bacteria increased to 10^6cfu/g after 12 days of storage in ice.

Storage temperature might be associated with the variation of microflora (Shamshad *et al*, 1990). Shamshad *et al.* (1990) reported that mean aerobic plate count of fresh shrimp (*Penaeus merguiensis*), initially 5.0 x 10^5cfu/g, increased with time and temperature to 6.4 x 10^9cfu/g at 35°C after 24 h. The initial bacteria were 30% gram+ organisms but changed to predominantly gram - psychrotrophs at lower temperatures and to mesophiles at higher

temperatures. The difference in chilling method has effect on the difference in quality of shrimp. Huidobro *et al.* (2002) found an increase in the formation of TMA and biogenic amines in the lot with flake ice after 4 days of storage, while levels of TMA in the liquid ice batch remained constant.

Total viable microbial counts (TVC) and chemical tests, such as analysis of trimethylamine (TMA) and total volatile basic nitrogen (TVB-N), have been used for years in the seafood industry to evaluate spoilage of seafood (Gill, 1990). Limits for these quality indicators are defined in standards, guidelines, and specifications for acceptability. Similar acceptability limits have been used for shrimp as for fish. Limits of TVB-N of 30 mg N/100 g and TMA of 5 mg N/100 g in shrimp have been reported (Cobb *et al.,* 1973). However, the average TVB-N values for fresh crustaceans are often higher (Oehlenschläger, 1997). The initial TVB-N content of fresh shrimp tails from different batches of shrimp stored on ice ranged from 13.5 to 38.2 mg N/100 g (Cobb *et al.,* 1976). Limits of TVB-N of 30 mg N/ g and TMA of 5 mg / g in shrimp have been reported (Cobb *et al.,* 1973).

2.7 Changes in sensory property

Fish quality is assessed by sensory methods based on changes in appearance, odor, color, flavor and texture. Sensory methods are fast, simple, sensitive and objective, but they rely on human judgement and proper training of panels (Strachan and Nicholson, 1992). High pass quality of shrimp as described by U.S.FDA includes translucent for appearance, fresh ocean air for odor, firm texture and sweet in taste (U.S. Food and Drug Administration, 2005). Variations in freshness on the first day may be due to several factors such as onboard handling of fish, fishing techniques, initial number of bacteria of fish, season of fishing etc. (Botta *et al.,* 1987). Manthey *et al.* (1988) reported that quality deterioration was noticed somewhat later in cooked samples than in the raw fish. Changing in fresh water prawn *Macrobrachium rosenbergii* and *Penaeus monodon* were investigated under various storage conditions. Both species were found organoleptically acceptable for 6 days and delayed icing considerably shortened the shelf-life of the fresh prawn *Macrobrachium rosenbergii* (Kamal *et al.,* 2000). Yamagata and Low (1995) found that the overall appearance of shrimp was acceptable for 2 days of storage in ice, with only slight blackening in the gill regains. Huidobro *et al.* (2002) investigated the effect on the quality of deepwater pink shrimp (*Parapenaeus longirostris*) of onboard chilling with liquid ice versus

the traditional chilling with flaked ice; at the same storage time, shrimps stored in flaked ice had higher color score than that stored in liquid ice but the firmness score of shrimp stored in liquid ice was higher than that of stored in flaked ice. Zeng *et al.* (2005) showed a good correlation between quality indicators including microbial growth, TVC, pH, TVB-N, TMA-N, responses of an electronic nose and sensory evaluation.

3. Effect of thermal process on fish and shellfish quality

Thermal processing is one of the most effective means of preserving food (Karel *et al.,* 1975). Prior to consumption, shrimp meat stored for a certain period in frozen or chilled conditions needs processing such as heat-processing (Mizuta *et al.,* 1997). Thermal process causes the denaturation of muscle proteins and degree of denaturation varies with species. Fish myosin is very unstable in comparison with that of mammal (Ogawa *et al.,* 1994). Johnston *et al.* (1973) found a relationship between the thermal stability of fish myosin and the environment temperature in which the species live. The myosin of cod (*Gadus morha*) which lived in cold water was more labile, compared with that of snapper (*Lutjanus sebae*) which lived in tropical area (Davies *et al.,* 1988).

Shrimp meat is enhanced in firmness or solidity by heat processing and becomes too solid and unpalatable when its inner temperature is above 100°C. Japanese professional cooks are careful to keep the central inner temperature of prawn meat below 65 to 70°C (Mizuta *et al.,* 1999). The texture and taste of the product become rapidly undesirable with excessive firmness and a lack of juiciness once the heating temperature exceeds 70°C (Mizuta *et al.,* 1999).

After canning, shrimp lost more than 9% to 14% of its water content. The decrease in water content resulted in the relative increase in protein, fat, and ash content (Mohan *et al.,* 2006). Denaturation of the shrimp muscle during heat processing enhances the loss of water-holding capacity of shrimp meat (Murakami, 1994). Heat processing enhanced firmness and degree of shrinkage deformation of kuruma prawn *Penaeus japonicus* (Mizuta *et al.,* 1999).

Apparent relationships between total collagen content and raw meat firmness were reported for fish muscles (Sato *et al.,* 1986) and for crustacean muscles (Mizuta *et al.,* 1994). Sato *et al.* (1986) pointed out that the texture of cooked fish meat was affected somewhat by gelatin derived from the muscle collagen on the basis of the fact that cooked meats of species with high

collagen content tended to show more elastic texture than those of species with relatively low collagen.

Mohan *et al.* (2006) reported that thermal processing caused an increase in TVB-N and TMA-N values in both canned and pouched products but the increase in TMA-N content in the pouch was much less compared to raw shrimp. This increase could be due to the breakdown of proteins, amino acids, and other nitrogenous compounds such as trimethylamine oxide, nucleic acids, and amines (Chia *et al.,* 1983). The yield of cooked meat under commercial conditions depends on the size and age of the shrimp (Erdogdu *et al.,* 2004). Small shrimp yield less cooked meat through processing, compared with the larger shrimp (Crawford, 1981). The reduction of process time resulted in an increase had beneficial effects on the quality parameters, sensory and textured parameters in heated process shrimp (Mohan *et al.,* 2006).

4. Effect of freeze-thawing process on fish and shellfish quality

Freezing and storage in the frozen state is an effective method for preventing microbial spoilage of foods. However, changes in the functional properties of protein of animal origin have been observed (Wagner and Anon, 1986). The protein denaturation, in particular myofibrillar proteins, is the main quality deterioration during frozen storage (Zayas, 1997). Badii and Hawell (2001) observed that frozen storage of cod and haddock muscle at 10°C led to the denaturation of myosin, causing a reduction in solubility and selective precipitation of proteins. Extended frozen storage caused the severe changes in tertiary conformation of actomyosin. As a result, an exposure of the interior in molecule occurred, leading to an increase in surface hydrophobicity. The formation of disulfide bonds via oxidation of SH groups or disulfide interchanges is coincidental with the decrease in total and surface SH contents (Hayakawa and Nakai, 1985).

Freezing and thawing also affect the membrane structures of muscle tissues. Normally enzymes in fresh tissue are retained in intracellular organelles. The leaked enzymes are regarded as markers of membrane damage and the activity of lysosomal enzymes in the centrifuged tissue fluid has been used to differentiate frozen from fresh fish (Rehbein, 1988). Membrane integrity was estimated as the volume of centrifuged tissue fluid (CTF) and by lysosomal β-N-acetyl-glucosaminidase (NAG: E.C.3.2.1.30) activity in CTF (Nilsson and Ekstrand, 1995).

The disintegration of membrane structures increased as the number of freeze-thaw cycles increased. Benjakul and Bauer (2001) reported that when the number of freeze-thaw cycles of cod and catfish increased, the activities of α-glucosidase (AG:E.C.3.2.1.20) and β-N-acetyl-glucosaminidase increased. Ca^{2+}-ATPase activity can be used as an indicator for integrity of the myosin molecule. Ice crystals and the increase in ionic strength of the system during frozen storage caused myosin denaturation and disruption of the actin-myosin comples, as indicated by the decrease in Ca^{2+}-ATPase activities (Benjakul and Bauer, 2000). Ang and Hutin (1989) reported that crosslink of cod myosin stored at -25°C and -80°C was related to the decrease in ATPase activity. The loss of Ca^{2+}-ATPase increased with increasing freeze-thaw cycles. Srinivasan *et al.* (1997) reported that the decrease in shear force of *Machrobrachium rosenbergii* was found after 3 cycles of freeze-thawing process.

Extended frozen storage caused the severe changes in tertiary conformation of actomyosin. The increase in surface hydrophobicity possibly caused the association of hydrophobic groups via hydrophobic interaction. The denaturation and aggregation of protein started from the formation of disulfide bond, followed by a rearrangement of hydrophobic and hydrogen bonded regions on an intra- and inter-molecular basis (Buttkus, 1974). Badii and Howell (2002) reported the initial increase in surface protein hydrophobicity of cod muscle in the first month before decreasing during the frozen storage at -10 and -30 °C. The level of reactive SH groups decreases considerably during frozen storage. This demonstrates that more disulfide bonds are formed in the muscle during frozen storage (Zayas, 1997). Disulfide bridges are the important covalent bonds, which relates to aggregation of protein (Sikorski *et. al.,* 1990b). The formation of disulfide bonds via oxidation of SH groups or disulfide interchanges was coincidental with the decrease in total and surface SH contents (Hayakawa and Nakai, 1985). SH groups located in the head portion of myosin have been found to play an important role in ATPase activity.

OBJECTIVES

1. To study chemical composition and physical/thermal properties of black tiger shrimp and white shrimp muscle
2. To monitor the changes in physicochemical properties and microstructure of black tiger shrimp and white shrimp muscle induced by heating and freeze-thawing process
3. To investigate the quality changes of black tiger shrimp and white shrimp muscle with different pretreatments and icing methods during extended storage.