CHAPTER 1

INTRODUCTION

Gelation of surimi proteins is a process involving the unfolding and aggregation of proteins. During heating, the proteins unfold, exposing reactive groups, in which intermolecular bonds can be formed with neighboring protein molecules. When sufficient bonding occurs, a three-dimensional network is developed, resulting in a gel (Lanier, 2000). Salt is an important element for the formation of meat gels structure (Chang *et al*., 2001). However, the reduction of sodium chloride results in the decreased water-binding capacity. Polyphosphates can sometimes be used to replace sodium chloride (Chang and Regenstein, 1997). Phosphate is normally added to surimi in combination with cryoprotectants such as sugar or sorbitol (Sultanbawa and Li-Chan, 2001). Furthermore, proteolysis is associated with the losses in seafood quality during handle and storage (Jiang *et al*., 1991; Benjakul *et al*., 2003). To improve the gel properties, some additives have been used in surimi and surimi products. Some protein additives have been added into surimi to alleviate protein degradation caused by the endogenous proteinases. Bovine plasma protein (BPP), porcine plasma protein (PPP), egg white (EW) and potato powders can be used as food grade inhibitors in surimi (Lee *et al*., 2000; Benjakul *et al*., 2001). Additionally, TGase is able to catalyze the formation of ε-(γ-glutamyl) lysine crosslinks and strengthen the gel (Seki *et al*., 1990; Sakamoto *et al*., 1995). TGase from microorganism such as *Streptovercillium mobaraense* has become popular to increase the gel strength by inducing the polymerization of protein (Washizu *et al*., 1994; Huang *et al*., 1992). Starch and other hydrocolloids are also used in surimi or surimi seafoods. They modify texture, improve freeze-thaw stability in the case of modified starch, and decrease the product cost (Lee *et al*., 1992). Furthermore, ι-carrageenan markly increased gel strength when synergistic compounds such as Ca^{2+}- bridge - forming agent were used (Bullens *et al*., 1990; Lee and Chung, 1990). Bullens *et al* (1990) reported that the surimi gel strength can be significantly improved when carrageenan is used at the level 0.25% with whey protein concentrate. Due to the tougher texture with an excessive drip loss of fish gel products such as fishball after freezing and frozen storage, the

losses in quality and acceptability generally occur. Positive result of some hydrocolloids and gum to control of freeze-induced texture changes has been reported (Foegeding and Ramseym, 1987; Yang and Park, 1998).

So far, a few studies have been performed on gel from invertebrate meat (Jaczynski and Park, 2002). These differences are thought to derive from differences in the intrinsic nature of proteins, such as the presence of paramyosin, content of myosin, and the action of endogenous enzymes, particularly proteinases and transglutaminase, in the thermal gelling processes (Sano *et al*., 1986; Park *et al*., 2003; Yoshida *et al*., 2003; Park *et al*., 2005). Generally, shrimp meat has poor gel forming ability, which is possibly associated with low setting phenomenon or the inappropriate protein structure for cross-linking or gelation (Thammatinna *et al*., 2007). Therefore, the improvement of gel from shrimp meat should be focused by lowering the autolytic reaction and enhancing the setting phenomenon. Additionally, to maintain the gel properties of shrimp gel products during frozen storage, some selected starch or hydrocolloid should be used. As a consequence, the prime quality of shrimp gel can be obtained after frozen storage.

Literature Review

1. Muscle protein composition

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 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 (Pearsons and Young, 1989; Mackie, 1994). 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. Sarcoplasmic proteins also include enzymes influencing the quality of fish. Enzymes of the glycolytic pathway and the hydrolytic enzymes of the lysosomes are found to be important (Sikorski *et al.,* 1990).

1.2 Stroma proteins

The stroma is composed of 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).

1.3 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 1 (Ashie and Simpson, 1997).

1.3.1 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

Table 1 Contractile proteins in food myosystems

Proteins	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)

myosin alone, and 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 is responsible for its association into thick filaments (Foegeding *et al.*, 1996).

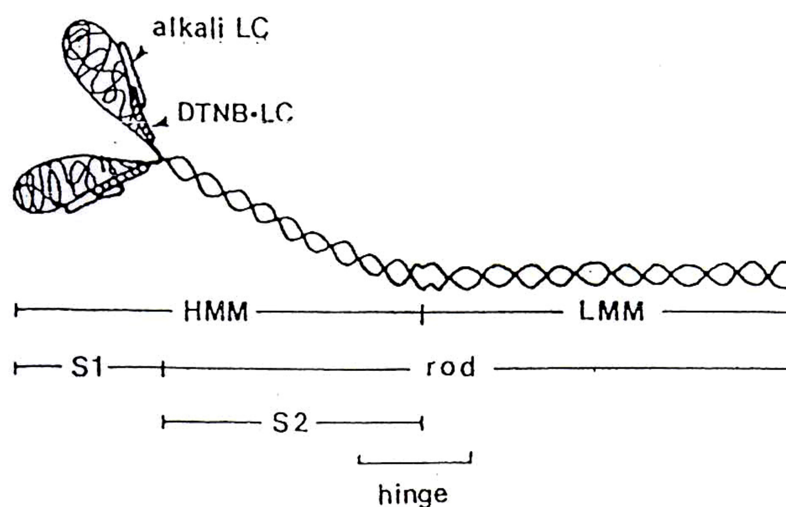

Figure 1 Model of myosin molecule

Source: Xiong (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%), arginine (12%) and lysine (9%), but low in proline content. Paramyosin, a rod-shaped

alpha-helical chain, 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).

1.3.2 Actin

Actin is about 15 to 20% of myofibrillar protein (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).

1.3.3 Actomyosin

When actin and myosin are mixed *in vitro*, a complex, call actomysin, is formed. This complex can be dissociated by addition of ATP. Actomyosin is the main state of actin and myosin in postmortem muscle because ATP is depleted by postmortem metabolism (Ochai and Chow, 2000).

1.3.4 Tropomyosin

Tropomysin, a rod-like molecule, consists of two polypeptide chains, each with a molecular weight range of 34,000-36,000 dalton, which associates 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).

1.3.5 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 2b).

2. Gelation of muscle protein

Gelation is important for meat product texture. Fish meat, when ground with salt, forms a viscous sol. Upon heating, sol turns into a viscoelastic gel. The rheological characteristics of the gel depend on the properties of the myofibrillar proteins, which are affected by the species and freshness of the fish, as well as on the processing parameters, mainly protein concentration, pH, ionic strength and temperature (Niwa, 1992). Generally, myosin alone forms excellent gels. Actin has a synergistic or antagonistic effect on myosin gelation, depending upon the myosin / actin ratio in the gelling system (Grabowska and Sikorski, 1976; Matsumoto, 1980). The process of muscle protein gelation involves two steps, denaturation and aggregation (Ziegler and Aton, 1984).

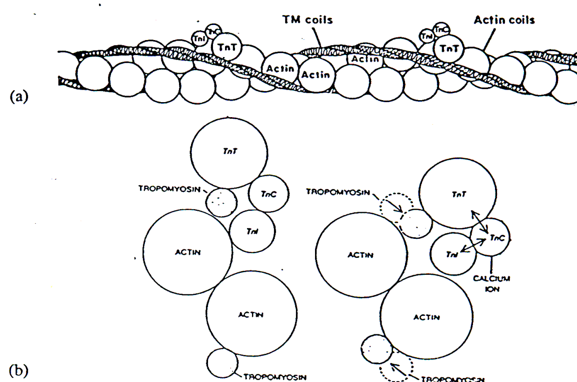

Figure 2 Thin filament of muscle formed by the filament of tropomyosin molecules wound in each of the two grooves of the actin helix (a) and proposed model for configuration of actin, tropomysin and troponin (Tn) subunits (b).

Tn T = troponin-tropomyosin subunit

Tn I = troponin-inhibitory subunit

Tn C = troponin-calcium-binding subunit

Source: McCormick (1994)

2.1 Protein denaturation

Addition of salt and heating are two major factors involved in denaturation and gelation of muscle proteins. Yamamoto *et al* (2002) reported that heating temperature of 30-40°C and KCl concentrations of 0.1-0.5 M caused the denaturation of carp myosin. In 0.1 M KCl, heating temperature affected the denaturation of rod more significantly than subfragment-1 (S-1), and a slow decrease in solubility at 30°C was accompanied by a slow denaturation of rod. Increased KCl concentrations reduced the rod denaturation rate at 40°C, but increased the denaturation rate at 30°C. At concentrations above 0.3 M KCl, the denaturation rate for rod become identical to that for S-1 at both temperatures. Table 2 gives a summary of changes, which may occur during the heat denaturation of actomyosin.

Table 2 Conformation changes occurring during the thermal denaturation of natural actomyosin

Temperature (°C)	Protein (s) or segment involved	Description of events
30-35	Native tropomyosin	Thermal dissociation from the F-actin backbone
38	F-actin	Super helix dissociates into single chain
40-45	Myosin	Dissociated into light and heavy chains
	Head	Possibly some conformational change
	Hinge	Helix to random coil transformation
45-50	Actin, myosin	Actin-myosin complex dissociates
50-55	Light meromyosin	Helix to coil transformation and rapid aggregation
>70	Actin	Major conformational changes in the G-actin monomer

Source: Ziegler and Aton (1984)

2.2 Aggregation

Denatured proteins undergo aggregation noncovalently to form a fine elastic network when surimi sol is subjected to heating process (Figure 3). Myosin is a predominant protein responsible for gelation of muscle. During heating of surimi pastes, the proteins unfold and intermolecular bonds are formed, leading to the cross-linking (Lanier, 2000). The number and kinds of interactions or bonds are not only affected by the species from which the surimi is derived (Suzaki, 1981; Shimizu, 1985) but also the heat condition in which the gel is made (Ishikawa, 1978; Akahane and Shimizu, 1990; Yamazawa, 1990).

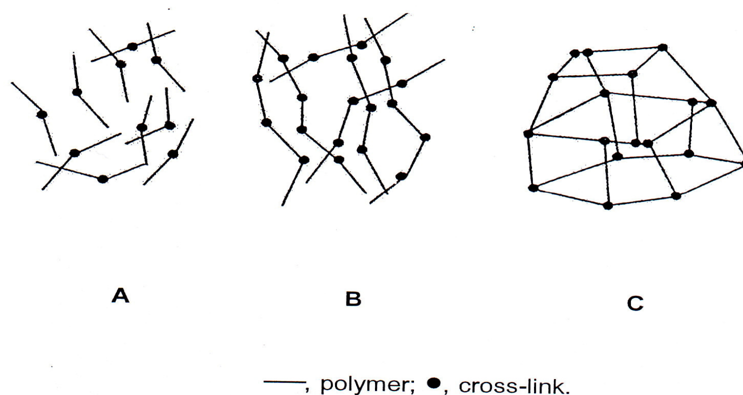

Figure 3 Formation of a gel network structure

Source: Niwa (1992)

The heat-induced gelation of myosin can be represented by two reactions. One is the aggregation of the globular head portion of the molecule which is complementary to and closely associated with the oxidation of SH groups and the other is the inevitable network formation by the thermal unfolding of the helical tail portion (Samejima *et al.*, 1981). Aggregation of myosin during heating is solely due to the association of the head portions (Samejima *et al.*, 1981). In the sol state, the rod chains have 100% helix-coil transition, which is sufficient to provide cross-links for a continuous network. The unfolding tends to prevent the gel from becoming progressively more tightly cross-linked. Chan *et al* (1993) reported that thermal aggregation of fish myosin was coincidental with an increase in the surface hydrophobicity of the unfolded domains of myosin molecules and was affected by the temperature at which these domains unraveled. Temperature and ionic strength have a profound effect on the hydrophobic

interaction. Both HMM and LMM are involved in the thermal aggregation of cod and herring myosin. Thermal aggregation may be initiated by the unfolding and interaction of HMM S-2, and further aggregation may be mediated through the interaction of LMM to form clusters of aggregate at higher temperatures (Figure 4).

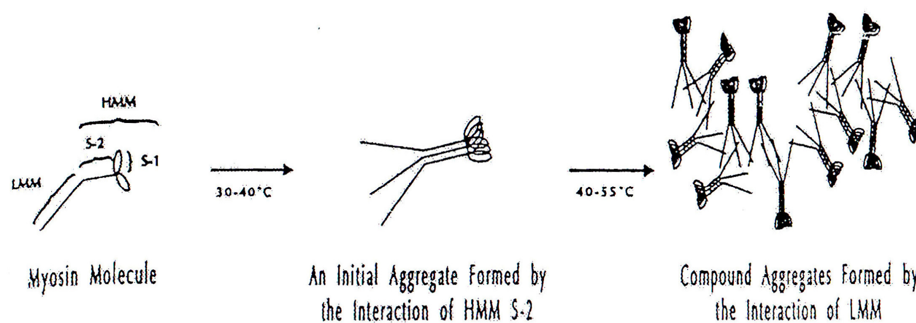

Figure 4 A schematic representation of the thermal aggregation of fish myosin.

Source: Chan *et al* (1993)

However, heating rate and / or time of heating affect the unfolding and appear to influence the gel formed (Foegeding *et al*., 1986). Excessive heating of the protein sol to a degree far higher than needed for denaturation leads to a metasol state which does not set into a gel upon cooling (Damodaran, 1989; Oakenfull *et al*., 1997). This may be related to β-elimination of disulfide bonds and scission of peptide bonds, which involves aspartate residues at high temperatures (Damodaran, 1989).

Roussel and Cheftel (1990) reported that gels with the highest texture characteristics were obtained when the surimi sol was incubated at 37°C or 4°C and then cooked at 90°C for 50 min. Prior incubation at 37°C, rather than at 4°C appeared to give kamaboko gels with the highest gel strength. Gel elasticity and water retention capacity were maximum after incubation at 37°C and gel rigidity and gel strength further increased upon cooking. Thus an elastic gel network may form at 37°C (or 4°C) from myofibrillar proteins partly solubilized and unfolded by NaCl, while gel strengthening during subsequent cooking may result from enhanced hydrophobic and other interactions.

Yongsawatdigul and Park (1996) reported that shear stress of Alaska pollock surimi gels with and without beef plasma protein (BPP) increased as heating rate decreased, but

shear strain was unaffected. An increase in shear stress was accompanied by an increase of cross-linked myosin heavy chain. Slow heating rates increased proteolysis in Pacific whiting surimi as shown by degradation of myosin heavy chain and low shear stress and shear strain. Proteolysis of whiting surimi with the addition of BPP was lessened to a greater extent at rapid heating rates (20 and 30°C/min) than at slow heating rates (1 and 5°C/min).

3. Proteolytic enzyme

Proteinase is the generic name given to those enzymes hydrolyzing the peptide bond in proteins and some synthetic substrates and coded as the EC 3.4.11-99. Proteinases, including peptidases and proteinases, are polyfunctional enzymes catalyzing the hydrolytic degradation of proteins (Garcia-Carreno and Hernandez-Cortes, 2000).

3.1 Classification of proteinases

Proteinases may be classified based on their similarities to well characterized proteinases, as trypsin-like, chymotrypsin-like, etc., their pH activity profiles as acid, neutral or alkaline proteinases, substrate specificity and mechanism of catalysis (Haard and Simpson, 1994). Additionally, proteinases are classified according to their source (animal, plant, microbial), their catalytic action (endopeptidase or exopeptidase) and the nature of the catalytic site. In EC system for enzyme nomenclature, all proteinases belong to subclass 3.4, which is further divided into 3.4.11-19, the exopeptidases and 3.4.21-24, the endopeptidases or proteinases (Nissen, 1993). Endopeptidases cleave the polypeptide chain at particularly susceptible peptide bonds distributed along the chain, whereas exopeptidases hydrolyze one amino acid from N terminus (amino peptidases) or from C terminus (carboxypeptidases) (Figure 5).

3.1.1 Endopeptidases

The four major classes of endopeptidases are serine proteinases (EC 3.4.21), cysteine proteinases (EC 3.4.22), aspartic proteinases (EC 3.4.23) and metalloproteinases (EC 3.4.24) (Nissen, 1993). Serine, cysteine and aspartic proteinases have serine, cysteine and aspartic acid side chains, respectively, as a part of the catalytic site. Modification or blocking of this side

chain usually leads to the complete inactivation of the enzyme and is a standard way of determining the nature of an unknown proteinase (Nissen, 1993).

The serine proteinases have maximal activity at alkaline pH, while the closely related cysteine proteinases usually show maximal activity at more neutral pH values. The aspartic proteinases generally have maximal catalytic activity at acidic pH. Among the digestive enzymes, pepsin, an aspartic proteinase, is secreted in the stomach and the serine proteinases, trypsin and chymotrypsin, are excreted in the duodenum, in accordance with pH values of the digestive tract (acid in the stomach and alkaline in the gut) (Nissen, 1993). The metalloproteinases contain an essential metal atom, usually Zn and have optimal activity near neutral pH. Ca^{2+} generally stabilizes these enzymes and the strong chelating agent, such as EDTA inhibits the activity (Nissen, 1993).

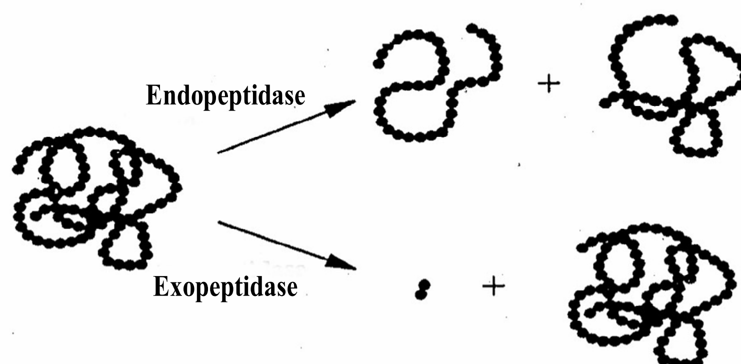

Figure 5 Action of endopeptidase and exopeptidase on protein structure.

Source: An *et al*. (1996).

3.1.2 Exopeptidases

The aminopeptidases (EC 3.4.11) are ubiquitous, but less readily available as commercial products, since many of them are intracellular or membrane bound. Carboxypeptidases are subdivided into serine carboxypeptidases (EC 3.4.16), metallocarboxypeptidases (EC 3.4.17) and cysteine carboxypeptidases (EC 3.4.18) according to the nature of the catalytic site (Nissen, 1993).

Tissue proteinases have the detrimental effect on the quality of stored muscle foods due to the sustained action of endopeptidase and exopeptidase that are involved in the

complete breakdown of tissue protein (Goll *et al.*, 1983). Proteinases are responsible for significant quality changes in fresh shrimp and prawn (Baranowski *et al.*, 1984). Acitivity of alkaline proteinase and exopeptidase in shrimp was detected (Doke and Ninjoor, 1987; Kolodziejsk and Sikorski, 1996). Shrimp muscle alkaline proteinase showed the maximal activity at pH 8.0 and 60°C and was classified as serine proteinase. The shrimp exopeptidase cleaving amino acid napthylamides at pH 6.8 and 40 $^{\circ}$C exhibited the characteristic of amino peptidase (Doke and Ninjoor, 1987). Those endogenous proteinases are associated with the low gel-forming capacity of Pacific white shrimp muscle (Jiang *et al*., 1992; Cortes, *et al*., 1997). Furthermore, shrimp muscle is preferentially degraded by various types of endogenous proteinases (Doke and Ninjoor, 1987; Jiang *et al*., 1991; Whitaker, 1997).

4. Proteinase inhibitor

An enzyme inhibitor is any substance that reduces the rate of an enzyme catalyzed reaction (Whitaker, 1994). Proteinase inhibitors mimic the protein substrate by binding to the active site of the proteinase. Specific inhibitors are active-site-direct substrate and combined with the catalytic or substrate-binding site of the enzyme to form a stable complex (Salvesen and Nagase, 1989). Nonspecific inhibitors are rare in nature, and the only one known is α_2-macroglobulin (α_2M) in plasma protein (Barrett and Starkey, 1973). Chelators that remove cations from metal-dependent proteinases, and the denaturant that alter catalytic sites are known as inactivators rather than inhibitors (Garcia-Carreno, 1996).

4.1 Natural proteinase inhibitor

Inhibitors have been isolated from a variety of organisms including bacteria, animal and plants. Their sizes are also extremely from 50 residues (e.g bovine pancreatic trypsin inhibitor) to up to 400 residues (e.g alpha-1 proteinase inhibitor). They are strictly class-specific except proteins of α_2M which bind and inhibit most proteinases through a molecular trap mechanism. Table 3 gives a list of organisms producing proteinase inhibitor (Garcia-Carreno *et al*., 1996).

Proteinase inhibitor commonly accumulates in high qualities in plant seeds, bird eggs and various body fluids. Proteinase inhibitors are also found in mammalian and marine

animal blood plasma, where they account for more than 10% of total protein (Ylonen *et al*., 1999; 2002; Tahtinen *et al*., 2002). Furthermore, plant seed such as legumes contains proteinase inhibitor which can be used to inhibit biological systems (Garcia-Carreno *et al*., 1996).

Table 3 Selected sources of proteinase inhibitors

Organism	Inhibitor	Characteristic	Affected enzyme
Microorganisms			
Actinomycetes	Leupeptin	Tripeptide	Calpain,cathepsin B, H and L and chymotrypsin
Streptomyces testaceous	Pepstatin	Pentapeptide	Aspartic proteinase: pepsin cathepsins and HIV-1
Plant			
Soybean	NA	Protein	Trypsin
Lima bean	NA	Protein	Trypsin
Verterbrate organs and tissues			
Plasma	a_2M	Protein	All classes
Pancreas	Aprotinin	Protein	Trypsin
Egg white	Ovomucoid	Protein	Serine protease

Source: Garcia-Carreno and Hernandez-Cortes (2000).

4.2 Classification of proteinase inhibitors

Proteinase inhibitors can be broadly separated into two general categories based upon their spectrum of activity: the nonspecific proteinase inhibitors and class-specific proteinase inhibitors (Whitaker, 1994). An inhibitor can react directly with essential groups of the active site of the enzymes or with specific groups on the enzyme not involved in the active site (Whitaker, 1994).

4.2.1 Cysteine proteinase

These inhibitors show the protective effect against cysteine proteinase released into circulation after cell death (Abrahamson *et al*., 1991). The cystatin superfamily contains three families of proteins that are related functionally as cysteine proteinase inhibitors and

evolutionarily by their amino acid sequence identity. These inhibitors occur in all cells and body fluids of mammals and many lower organisms. The affinity of the cystatins to the lysosomal cysteine proteinase is very high. They do not react with serine or other types of proteinases (Abrahamson *et al*., 1991).

4.2.2 Serine proteinase inhibitor

Serine proteinase inhibitor comprises the largest superfamily of the class-specific proteinase inhibitors. A feature of all the proteinase inhibitors in the serine superfamily in a particular peptides bond, located in a C-terminal domain, that is susceptible to attack by serine proteinase (Carlson, 1996). These inhibitors are very abundant in mammalian plasma and plant cells and play a main role in many physiologic processes (Otlewski *et al*., 1999).

Proteinase inhibitors isolated from soybeans are of two types: the Kunitz trypsin inhibitor (TI) and the Bowman-Birk (BB) inhibitor. The first group has an MW between 20 and 25 kDa, with a specificity directed primarily toward trypsin. The inhibitor combines tightly with trypsin. The BB inhibitor is capable of inhibiting both trypsin and chymotrypsin at independent reactive sites. BB inhibitor has a stable conformation even after disulfide bonds are broken by heating (Kennedy, 1998). Benjakul *et al* (2000) reported that porcine plasma protein contained serine protease inhibitor with a molecular weight of 60-63 kDa.

4.2.3 Aspartic proteinase inhibitor

The best characterized aspartic proteinase from mammals (pepsin, chymosin, cathepsin D and rennin) are all inhibited by pepstatin A. Aspartic proteinase inhibitors can be found in many sources such as potato, yeast, the nematode *Ascaris*, and squash (Garcia-Carreno and Hernandez-Cortes, 2000). These aspartic proteinase inhibitors from a multigene family consist of at least 10 members (Ritonja *et al*., 1990). These inhibitors are similar to the soybean trypsin inhibitor family, which also possesses trypsin inhibitory activity. An inhibitor from squash phloem exudates (Christeller *et al*., 1998) has no similarity with any other known protein, which suggests that it belongs to a new inhibitor family Squash. 2-macroglobulin was reported to be the major inhibitor of aspartic proteinase (Thomus *et al*., 1989).

4.2.4 Metalloproteinase inhibitor

Any substrate that complexes and/or removes an essential cation from an apoenzyme will be an inhibitor of that enzyme (Whitaker, 1994). Most inhibitors of metalloproteinase are able to chelate or bind the catalytic zinc atom. Synthetic inhibitors, therefore, commonly contain a negatively-charged moiety to which attach a series of other groups designed to fit the specificity pockets of a particular proteinase (Whitaker, 1994).

4.3 Application of proteinase inhibitors in food processing

Gel strength has been known to be affected by endogenous proteinases. Metalloproteinase was a major proteinase contributing to softening of gel from squid muscle (Nagashima *et al*., 1992). To alleviate the gel weakening caused by heat-activated proteinase, protein additives possessing the inhibiting activity towards proteinase such as bovine plasma proteins (BPP), egg white (EW), whey proteins, potato extract, etc. have been used to prevent the protein degradation and to improve the quality of gel (Wasson *et al* 1992; Reppond and Babbitt 1993). Furthermore, porcine plasma protein and chicken plasma protein have been reported to inhibit the autolysis and improve surimi gel properties (Visessanguan *et al.*, 2000; Benjakul *et al*., 2004a; Rawdkuen *et al*., 2004). However, additives showed the differences in autolysis inhibition. Morrissey *et al.* (1993) reported that BPP and potato extract exhibited higher inhibitory activity in Pacific whiting (*Merluccius productus*) surimi than did EW. However, Visessanguan *et al* (2000) reported that PPP interfered with the formation of actomyosin gel, resulting in the decreases in the rate and the magnitude of G'. Blood plasma has 3 main protein components, albumin, globulin (α, β, and γ), and fibrinogen (Raeker and Johnson 1995). These protein components have different structural, physical, and functional properties. When PPP is mixed in actomyosin and heated, unless it can enhance any type of interaction that can favor more ordered protein- protein interactions, it will interfere with the formation of a three dimensional gel network between myosin molecules. Benjakul *et al* (2004) reported that PPP was effective in increasing breaking force and deformation of kamaboko gels set at 40°C for 30 min and heated at 90°C for 20 min. The optimum level of PPP were 0.5, 0.5, 1.5 and 1.5 g/100 g for bigeye snapper, bigeye crocker, threadfin bream and barracuda surimi. In addition, Yongsawatdigul and Piyadhammaviboon (2004) reported that EW improved gel forming ability of lizardfish surimi to a greater extent than

WPC. Addition of 1% EW and pre-incubation at 25°C resulted in the increase of higher molecular weight cross-linked proteins. Although food grade inhibitors can enhance gel strength of surimi, they may have negative effects on quality, thus limiting their applications in surimi. BPP addition led to the lowered whiteness of the resulting gel (Akasawa *et al*., 1993). EW has a high cost and an undesirable egg-like odor at level required for inhibition (Porter *et al*., 1993). WPC are the inhibitors of choice regarding their commercial availability and a potential control of autolysis during processing (Weerasinghe *et al*., 1996).

5. Phosphate compound

Phosphates are compound prepared from phosphoric acid where the acid has been partially or fully neutralized with alkaline metal ions, predominately sodium, potassium, or calcium (Dziezak, 1990).

5.1 Classification of phosphates

5.1.1 Orthorphosphate

Orthorphosphate consists of one phosphorus atom tetrahedral surrounded by four oxygens. It can form straight-chain and cyclic polymers. These conpounds have three valences that can be filled by hydrogen atom, alkaline metal cations, or a combination of hydrogens and metal cations. Monobasic orthophosphates have one alkaline metal ion and two hydrogen; dibasis orthophosphate have two metal ions (Dziezak, 1990).

5.1.2 Condensed phosphate

Condensed phosphates are produced by heating mixtures of orthophosphates under controlled conditions. They are composed of two or more phosphorus atoms linked through a shared oxygen. This group includes strength-chain phosphates called polyphosphate and rings, termed metaphosphates (Dziezak, 1990).

Of the polyphosphates, pyrophosphates are the simplest as they have a two-phosphate chain. Tripolyphosphates are next in the series with three phosphorus atom and are followed by long-chain polyphosphates, which have four or more phosphorus atoms. Pyrophosphates and tripolyphosphates are crystalline materials unlike long-chain polyphosphates,

which are amorphous or glassy. Long-chain polyphosphates are not pure compounds but instead mixtures of many polyphosphate or varying chain lengths (Ellinger, 1997). Selection is based on their average chain length. Polyphosphate can be hydrolyzed, yielding increasing amounts of orthophosphates, pyrophosphates, and tripolyphosphates (Ellinger, 1972a). The metaphosphates are pure crystalline compounds composed of six-or eight-membered rings. Presently there are two metaphosphates, sodium trimetaphosphate and sodium tetrametaphosphate; only the first is used commercially (Dziezak, 1990).

5.2 Chemical properties and functionality of phosphate

Certain chemical properties of phosphate enable these compounds to produce a wide variety of effects in food products

5.2.1 Inactivation of metal ions

The function of phosphate is metal chelator (Park, 2000). The phosphates can inactivate metallic ions, which are capable of interfering with necessary food-processing reactions. They inactivate the metallic ion either by precipitating and removing them from interference with the desired food-processing reactions or by complexing and maintaining them in a soluble state (Ellinger, 1975). Very weak, soluble complexs are formed with alkaline-metal and ammonium ions. More stable but somewhat dissociated complexs are form with alkaline – earth metals, such as calcium, magnesium, etc. Very stable, soluble complex are formed with the transition metal ions, such as copper, nickel and iron. A significant advantage in the use of phosphates to complex nutritionally important ions, such as calcium, magnesium, and iron, is that the ions can still be absorbed through the intestinal walls and utilized by the body. Their absorption and reaction may actually be increase in the form of their complexes (Ellinger, 1975). The polyphosphate anion can bind calcium more firmly than sodium; therefore, sodium pyrophosphates exchange their sodium ions for calcium ions when they are present.

5.2.2 Complexing organic polyelectrolyte food constituents

In solution, phosphates are polyvalent anions, as they have more than one negative charge. Orthophosphates have up to three negative charges depending on the pH, and

polyphosphates can be even more anionic. As a result of their highly charge nature, polyphosphates interact with various food constituents to produce many useful effects (Van Wazer, 1971). They can become adsorbed onto surfaces of certain constituents and affect the surface charge, thereby promoting deflocculation, dispersion, emulsification, or suspension of the constituent (Van Wazer, 1971). The ability of polyphosphates to attach themselves to positively charged sites of large molecules such as proteins thus increases water-binding and gel formation of the proteins, improves whipping properties (by increasing the solubility of protein), and improves the precipitation and insolubilization of proteins for separation (Ellinger, 1972b; Van Wazer, 1971). The polyelectrolyte properties of polyphosphates generally increase with chain length (Ellinger, 1972b).

5.2.3 Buffering or pH stabilization

The ability to maintain a constant pH after addition of acids or bases is termed "buffering". For the pH ranges of 2 to 3, 5.5, to 7.5 and 10 to 12, the orthophosphates such as mono- and disodium phosphates and the pyrophosphates such as sodium and pyrophosphates such as sodium acid pyrophosphate have the best buffering capacity (Van Wazer, 1971). Long-chain polyphosphates are generally poor buffers, and their buffering capacity decrease with increasing chain length. Phosphate can be used to increase or decrease pH to optimum levels. Both the acidic phosphate such as monosodium phosphate, monoammonium phosphate, and sodium acid pyrophosphate, and the alkaline phosphates such as di- and tetrasodium phosphates, sodium tripolyphosphate, and tetrasodium pyrophosphate are commonly used for this purpose (Dziezak, 1990)

5.3 Application of phosphate compounds in muscle foods

Phosphates have been used as essential additives in processed meat to improve the texture and to retain the juiciness, flavor and mouth feel. Increased myofibrillar / cytoskeletal protein extraction by phosphate in the presence of NaCl was associated with increased beef myofibrillar swelling and increased beef muscle water-holding capacity (Peterson *et al*., 1988). The effectiveness of phosphates on water retention properties of meat products depends on the type of phosphates, the amount used, and the specific food product (Lewis *et al*., 1986). The

effectiveness of phosphates on prevention of cook loss of meat products was in the following order: pyrophosphate > tripolyphosphate > tetrapolyphosphate > hexametaphosphate (Trout and Schmidt, 1986). Phosphates have been widely accepted as additive in fish and seafood because they improve functional properties during processing (Chang and Regenstein, 1997). Samejima *et al* (1985) found that both the protein extractability of non-heated myofibrils and the storage moduli of heat-induced gels were markly increased at high (0.3-0.6 M) concentrations of sodium chloride with the addition of pyrophosphate (plus magnesium chloride). Additionally, Julavittayanukul *et al*. (2006) reported that kamaboko and directly heated gels from bigeye snapper surimi added with 0.05% PP had the increases in breaking force and deformation by 17.35% and 11.52%, and 13.54% and 3.53%, respectively, compared with the control gel (without PP addition). PP, which has the lowest molecular weight, might distribute uniformly and solubilize or dissociate the actomyosin complex more effectively, compared to other phosphates used (sodium tripolyphosphate and sodium hexametaphosphate) and it exhibited a greater gel-strengthening effect than others, particularly at the appropriate concentration (Julavittayanukul *et al*., 2006). Knipe *et al* (1985) reported that the level of solubilized protein in raw meat emulsion increased with increasing portion of magnesium chloride. The sodium pyrophosphate was added to dissociate actomyosin into actin and myosin, while the purpose of the magnesium ion was to shift the isoelectric point of myosin to a value more favorable for solubilization at neutral pH (Sarkar, 1950). Pyrophosphate was used in the washed minced chicken muscle tissue to dissociate actin from myosin (Comissiong and Hultin, 1978). The improved solubility was obtained when 200 mM sodium chloride and 10 mM magnesium chloride and sodium pyrophosphate was used (Chang *et al*., 2001). Pyrophosphate dissociated the actomyosin and enhanced the thermal denaturation of Alaska pollack myosin at high salt concentration (Torigai and Konno, 1996). Benjakul *et al* (2007) found that ATPase activity of natural actomyosin (NAM) from kuruma prawn muscle treated with 5 mM PP decreased markedly when incubated at temperatures greater than 30°C, suggesting lowered thermal stability of the liberated myosin molecule. Moreover, in the presence of 5-10 mM $MgCl_2$, NAM treated with 5 mM PP underwent dissociation effectively. PP at high concentration (5 mM) induced the dissociation of the actomyosin complex, releasing myosin, which underwent denaturation easily.

6. Improvement of gel quality

6.1 Use of sodium chloride

Salts affect protein stability in two different ways. At low salt concentrations, ions interact with protein via non specific electrostatic interaction (Damodaran, 1996). This electrostatic neutralization of protein charges usually stabilizes protein structure. Complete charge neutralization by ions occurs at or below 0.2 ionic strength, and it is dependent on the nature of salt. However, at higher salt concentrations, salt has ion specific effects that influence the structure stability of protein. Protein structure is influenced more by anion than by cation (Damodaran, 1996). However, Rengenstein *et al* (1984) reported that cation have a greater effect than anion on water binding potential (WBP) and expressible moisture content (EM) of ground rainbow trout muscle. Furthermore, the addition of salt shifted the denaturation transition to lower temperature and decreased the enthalpies of heat denaturation (Park and Lanier, 1989). The addition of salt might cause a partial unfolding of proteins and increased sensitivity to denaturation (Park and Lanier, 1989). Gill *et al* (1992) concluded that thermal aggregation of herring myosin showed little dependence on salt concentration (0.6-1.4 M NaCl. pH 6.5) but salt enhanced the aggregation of cod myosin at heating temperatures higher than 50°C. Roussel and Oheftel (1990) found that adequate gel texture of kamaboko gel from sardine surimi require a NaCl content of 1.7-3.5%. Kubota *et al* (2006) reported that the maximum gel strength of walleye pollack surimi gel was maximal when the gel contained about 3%NaCl.

6.2 Use of calcium ion

Calcium compounds are commonly added as surimi gel enhancer (Lee and Park, 1998). During the setting, myosin is denatured and aggregated, and simultaneously polymerized by calcium depending endogenous TGase. TGase is responsible for setting phenomenon and enhances the surimi gelling properties in a great extent (Seki *et al*., 1998). Hemung and Yongsawatdigul (2005) reported that the unfolding of myosin and actin induced by $CaCl_2$ resulted in an exposure of free SH groups, which subsequently underwent disulfide interchanges. Similar effect of $CaCl_2$ on the formation of disulfide linkages and hydrophobic interactions were also found in α - crystallin molecules (Valle *et al*., 2002). The extent of disulfide bond formation at 40°C was greater than that at 25°C. It was speculated that disulfide bond might be partly

responsible for aggregation of myosin set at 40°C. Addition of $CaCl_2$ to fish protein paste induced hydrophobic interactions and disulfide linkages of myosin and actin at 40°C to a greater extent than at 25°C. Apart from ε-(γ-glutamyl) lysine isopeptide bonds catalyzed by Ca^{2+}-dependent endogenous TGase, hydrophobic interactions and disulfide linkages could be involved during setting of fish protein (Hemung and Yongsawatdigul, 2005). Benjakul and Visessanguan (2003) found that setting either at 25 or 40°C, prior to heating at 90°C, resulted in an increase in both breaking force and deformation of surimi from both species of bigeye snapper (*Priacanthus tayenus and Priacanthus macracanthus*). In addition, the breaking force and deformation of surimi from both species increased markedly with the addition of calcium chloride. Youngsawatdigul *et al* (2002) reported that breaking force of threadfin bream surimi gel was highest when 0.2% Ca^{2+} was added and set at 40°C than at 4 and 25°C because activity of TGase was greater at 40°C. Lee and Park (1998) found that addition of 0.2% calcium compounds improved shear stress of Pacific whiting surimi whereas the lower concentrations (0.05 to 0.1%) effectively increased gel texture of Alaska pollack. Optimum $CaCl_2$ was attributed to the varying inherent concentrations of Ca^{2+} in muscle among species (Lee and Park, 1998).

7. Transglutaminase

TGase is transferase, having the systematic name as protein glutamine γ-glutamyltransferase (EC 2.3.1.13). It catalyzes the acyl transfer reaction between γ-carboxyamide groups of glutamine residues in proteins peptides, and various primary amines (Motoki and Seguro, 1998). When the ε-amino group of lysine acts as acyl acceptor, it results in polymerization and inter- or intra- molecular cross-linking of protein via formation of ε - (γ - glutamyl) lysine linkages. This occurs through the exchange of the ε- amino group of the lysine residue for the ammonia at the carboxyamine group of a glutamine residue in the protein molecule(s) (Figure 6). In the absence of primary amines, water may act as the acyl acceptor, resulting in deamination of γ-carboxyamine. Formation of covalent cross-links between protein is the basis of the ability of TGase to modify the physical properties of protein food (Ashie and Linier, 2000).

TGase has a broad specificity for primary amine acceptors (peptide-bound lysine or polyamines) (Folk and Finleyson, 1997). In contrast, relatively few proteins contain glutamine

residues that form acyl- enzyme intermediates. This capability is influenced by the amino acid sequence (including charge) surrounding the susceptible glutamine residue and local secondary structures that are not well defined (Folk, 1980). Proteolysis of nonreactive protein can convert it to a transglutaminse substrate (Greenberg *et al*., 1991)

TGase activtity in tissue may be determine by one of several mechanisms including: amine incorporation into substrates using monodansylcadaverine, hydroxamate or radioactive putrecine (Folk and Cole, 1996; Folk and Chung, 1985), disappearance of amino group by trinitrobenzenesulfonate or fluorescence intensity methods (Ikura *et al*., 1980); increase in molecular weight of substrate by sodium dodecylsulfate-polyacylamide gel electrophoresis (SDS-PAGE) (Ikura *et al*., 1980); release of ammonia (Backer-Royer *et al*., 1992); or measurement of functional effects such as viscosity and gel strength (Sakamoto *et al*., 1994).

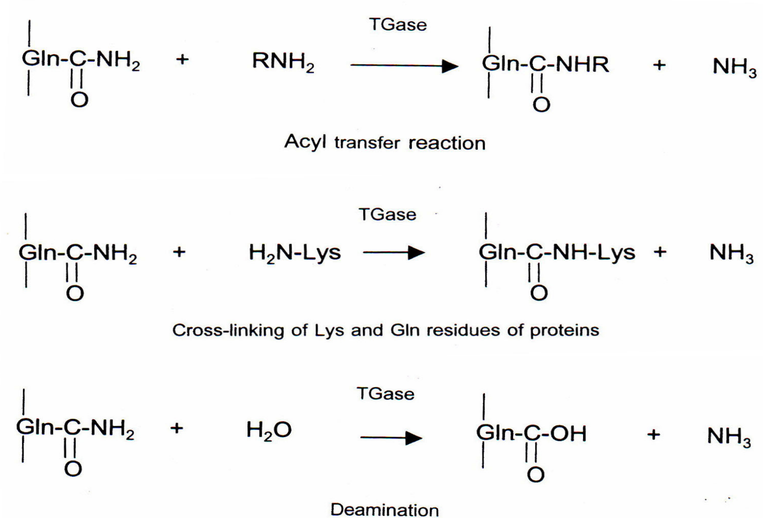

Figure 6 Reaction catalyzed by TGase

Source: Ashie and Lanier (2000)

Table 4 Sources and characteristics of some TGase

Sources	Mol.Wt (kDa)	Optimum Temp (°C)	Optimum pH
Mammal			
Human plasma factor XIII	300-500[t]		
Bovine factor XIIIa			
Guinea pig liver	75-85[m]		8.0
Rabbit liver	80[m]		
Plant			
Pea seedlings			
Alfalfa	39[m]		
Microbial			
Streptoverticillum mobaraense	40[m]	50	6.0-7.0
Physarum polycephalum	77[d]		
Streptoverticillium ladakanum	37.5	50	6.0
Seafood			
Rea sea bream liver	78	55	9.0-9.5
Carp muscle	80		
Walleye pollack liver	77	50	9.0
Lobster muscle	200		
Japanease oyster	84/90	40/25	8.0
Limulus hemocyte	86		
Scallop	80		
Botan shrimp	80		
Squid	80		
Rainbow trout	80		
Atka mackerel	80		

[t]tetramer; [m]monomer, [d]dimer

Source: Adapted from Ashie and Lanier (2000)

7.1 Source of transglutaminase

TGase has been found in tissue of various species including mammals, birds, fish and shellfish, microorganisms, and plants (Table 4).

7.1.1 Plasma transglutaminase (Factor XIII)

Role of plasma TGase of higher animal (also refer to as fribrinoligase or fraction XIIIa) has been well established as cross-linking of the fibrin clot hemostasis. The zymogen factor XIII is activated by thrombin, another component of plasma that also induces clotting of fibrinogen.

7.1.2 Liver transglutaminase

Mammal and fish livers were found to be the source of TGase, generally termed liver TGase. The molecular weight of pollack liver TGase was estimated to be 77 kDa from SDS-PAGE mobility under the reduced condition. This is similar to that of guinea pig liver (76.6 kDa) (Ikura *et al*., 1980), red sea bream liver TGase (78 kDa) (Yasueda *et al*., 1994) and partially purified carp muscle TGase (80 kDa) (Kishi *et al*., 1991). In contrast, the molecular weight of Ca^{2+}-independent microbial TGase was one half (38 kDa) (Ando *et al*., 1989) of that found in the tissue-type TGase of vertebrates. The concentration of Ca^{2+} to express full walleye pollack liver TGase activity was 3 mM. Thus, this TGase requires higher Ca^{2+} than red sea bream liver TGase liver, which requires 0.5 mM Ca^{2+} for its maximum activation (Kumazawa *et al*., 1996).

7.1.3 Microbial transglutaminase

TGase has been found in some microorganisms such as *Streptoverticillium mobaraense* (Gerber *et al*., 1994) *Streptoverticillium sp* (Ando *et al*., 1989) *Bacillus subtilis* (Ramanujam and Hageman., 1990) and *Streptoverticillium ladakanum* (Tsais *et al*., 1996). Since MTGase is excerted into the culture medium, cell disruption is unnecessary. Its purification thus proves to be rather easy. As a consequence, its commercialization has been accelerated (Kanaji *et al*., 1993). Physicochemical properties, such as molecular weight and secondary structures, and enzymatic properties have already been reported (Ando *et al*., 1989; Nonaka *et al*., 1989; Kanaji *et al*., 1993). The isoelectric point of MTGase was approximately 8.9. The molecular weight of

MTGase was previously determined to be 40,000 on both SDS-polyacrylamide electrophoresis (SDS-PAGE) and gel-permeation chromatography (Kanaji *et al*., 1993). MTGase has a molecular weight of 38,000 and comprises 331 amino acid residue (Figure 7) (Kanaji *et al*., 1993).

The pH optimum of MTGase was around 5 to 8. However, even at pH 4 or 9, MTGase still expressed some enzyme activity (Motoki and Seguro, 1998). MTGase is thus considered to be stable over a wide pH range. The optimum temperature for enzymatic activity was 50°C, and MTGase fully sustained its activity even at 50°C for 10 min (Motoki and Seguro, 1998). TGase, including the well-characterized guinea pig liver enzyme, requires Ca^{2+} for expression of enzymatic activity. However, MTGase from a variant of *Streptiverticillium mobaraense* is totally Ca^{2+}independent. Thus, MTGase is quite unique from other enzymes.

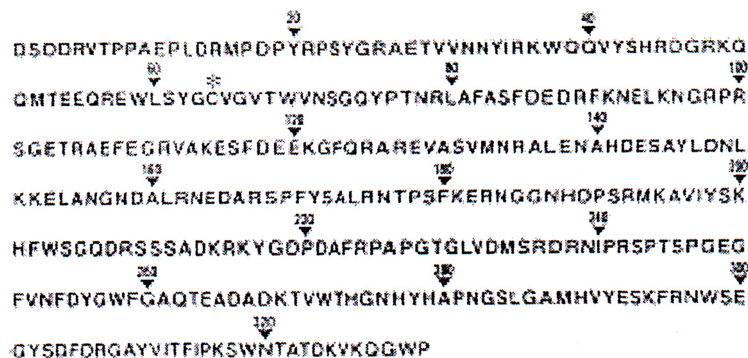

Figure 7 Primary structure of microbial transglutaminase (MTGase): all amino acids are denoted by the letter codes; *, indicates the possible active cysteine residue.

Source: Motoki and Seguro (1998)

MTGase is generally mixed with fish paste or surimi sol prior to setting. Jiang *et al* (2000) found that the optimum amounts of MTGase and setting conditions of surimi gel from threadfin bream and pollack surimi were 0.3 unit/g surimi either at 30°C for 90 min or at 45°C for 20 min for threadfin bream, and 0.2 unit / g surimi at 30°C for 60 min for pollack. SDS-PAGE analyses indicated that inter- and /or intra molecular cross-linkings were formed in the myosin heavy chain of MTGase containing surimi. Additionally, Tammatinna *et al* (2007) observed that setting at 25°C for 2 h could induce both endogenous and MTGase cross linking activity in Pacific white shrimp gel as evidenced by the increase in breaking force. Setting at 40°C showed the lower gel properties, compared with setting at 25°C. The unfolding of muscle

protein molecules at higher temperature might favor the aggregation via hydrophobic interaction. As a consequence, the reaction group for cross-lining induced by MTGase could be imbedded. Therefore, glutamine or lysine residues were possibly masked and cross-linking mediated by MTGase was impeded (Thammatinna *et al*., 2007).

To increase the efficacy of MTGase in gel strengthening, many approaches have been reported. Pressurization improved the mechanical properties of gel made from paste treated with MTGase and set at 25°C (Uresti *et al*., 2006). High pressure process modified protein structure and allowed MTGase to induce the cross-linking, thus improving texture attributes. The addition of MTGase caused the cross-linking of myosin heavy chain (MHC) and substantially increased the gel strength (from 536.6 to 2012.4 g x cm), while the recombinant cystatin could effectively prevent the MHC degradation and gel-softening during the production of mackerel surimi-based products (Hsieh *et al*., 2002). Combined uses of MTGase and recombinant cystatin revealed synergistic effectiveness on improving the quality of mackerel surimi (increase from 435 to 2438 g x cm). Jiang *et al* (2000) found that the combination of MTGase, reducing agent and proteinase inhibitor seemed to be a better way to improve gel-forming ability of hairtail surimi. The texture degradation caused by endogenous proteinase could be prevented by the inhibitors. The best condition for the improvement of gel forming ability was the combination of 0.35 unit MTGase / g, 0.1%$NaHSO_4$ and 0.01 mM E-64.

7.1.4 Endogenous transglutaminase

Endogenous TGase has been found in fish fresh. These enzymes induce the ε-(γ-glutamyl) lysine crosslinking in fish protein and have the impact on textural properties of fish sol during processing (Seki *et al*., 1990; Tsukamasa and Shimizu, 1990; Kimura *et al*., 1991; Sakamoto *et al*., 1995). Endogenous TGase is water soluble and can be removed if washing is too extensive (Nowsad *et al*., 1994). Youngsawatdigul *et al* (2002) reported that residued TGase activity of the first washed treadfin bream mince was lower than that of mince. TGase activities in fish muscles are shown in Table 6.

Total TGase activity differs depending on the measuring temperature (Tsukamasa *et al*., 2002). Optimum temperatures of TGase varied with species such as threadfin bream (55°C). (Youngsawatdigul *et al*, 2002), red sea bream (*Pagrus malor*) (55°C) (Yasueda *et*

al., 1994) bigeye snapper (*Priacanthus tayenus*) (40°C), bigeye snapper (*Priacanthus macracanthus*) (25°C) (Benjakul and Visassanguan, 2003) and Japanese oyster (29°C and 40°C) (Kumazawa *et al*., 1997). Differences in optimum setting temperature were also attributed to conformational changes of myosin (Joseph *et al*., 1994). In addition, the optimum $CaCl_2$ concentration for TGase also varied with the source of enzyme (Yasueda *et al*., 1994; Kumazawa *et al*., 1996). Worratao and Youngsawatdigul (2005) reported that optimum temperature and optimum pH of tilapia TGase were 37-50°C and 7.5, respectively. Tsukamasa *et al* (2002) reported that maximal TGase activity of carp meat (88.5 U/g sample) was found at 50°C, followed by threadfin bream (28.4 U/ g meat) at 40°C, white croaker (19.7 U/g meat) at 30°C and red sea bream (17.1 U/g meat) at 50°C. The addition of $CaCl_2$ and pre-incubation condition conditions significantly affect the textural properties of surimi gel (Yongsawatdigul *et al*., 2002). Benjakul *et al* (2003) reported that setting at either 25°C or 40°C before heating at 90°C increased both breaking force and deformation of surimi from bigeye snapper particularly when setting time increased. A decrease in solubility of surimi in a mixture of sodium dodecyl-sulfate, urea and β-mercaptoethanol suggested the increased formation of non-disulfide covalent bonding which coincided with increased gel-strength. Rawdkuen *et al* (2005) reported that addition of chicken plasma protein (CPP), $CaCl_2$ and thrombin in combination with setting effectively induced a large number of protein-protein interaction stabilized by nondisulfide covalent bond, which cannot be destroyed by the mixture containing sodiumdodeccyl-sulfate, urea and β-mercaptoethanol. Nondisulfide covalent bond formed might contribute to the strength of gel matrix.

Table 5 TGase activity level in the various fish muscles and surimi

Species	Activity level (unit/g muscle (wet weight))
White croaker	2.41
Carp	1.14
Sardine	0.83
Walleye pollack	0.41
Chum salmon	0.33
Alta mackerel	0.23
Rainbow trout	0.1
Wallleye pollack surimi	0.33
Chum salmon surimi	0.05

Source: Araki and Seki (1993)

8. Hydrocolloids and gums

Hydrocolloids are long chain polymers that dissolve or disperse in water to give a thickening or viscosity producing effect (Glickman, 1982). The water soluble polymers are still referred to as "gum", but this is gradually being replaced by the more scientific designation of "hydrophilic colloid", preferably "hydrocolloid" (Glicksman, 1982). Gum are not true colloids, but are rather polymers of colloids size (10A$^{\circ}$ - 1000A$^{\circ}$) (Glicksman, 1982). Gum particles are suspended in solution as colloids, therefore, gums are also known as hydrocolloids (Lee, 1997). Gums are effective as binding and texturing agents and provide structure, functionality, and desirable properties to fabricated foods. The important functionality of gums are viscosity or thickening, gelling, and freeze-thaw stabilization properties (Lee, 1997).

8.1 Starch

8.1.1 Chemical composition and properties

Starch is composed of two types of molecules, amylose and amylopectin. Amylose is a predominantly linear molecule of D-glucose units ranging from 250 to 2000 units joined by α-1,4 linkages, while amylopectin is a highly branched polymer of D-glucose units

linked by α-1,4 and α-1,6 bonds every 20-26 monomer units as shown in Figure 8 (Imeson, 1992). Amylose and amylopectin molecules are arranged radically and orderly within starch granules. The branches of amylopectin form both crystalline and amorphous regions in alternating layers with the amylose molecules occurring among the amylopectin molecules (Imeson, 1992). The major functional properties of starch are gelatinization and gel formation. When an aqueous starch solution is heated, the starch granules undergo the gelatinization process which is the disruption of molecular order within the granules. With continued heating, starch absorbs the surrounded water molecules resulting in the swelling of granules, leaching of amylose and eventual disruption of the molecules. When this final stage is reached, the process is called pasting. When hot starch paste is cooled, the dissolved materials become less soluble (referred to a retrogradation) and generally results in a viscoelastic, firm, rigid gel as depicted in Figure 9. (Glicksman, 1969).

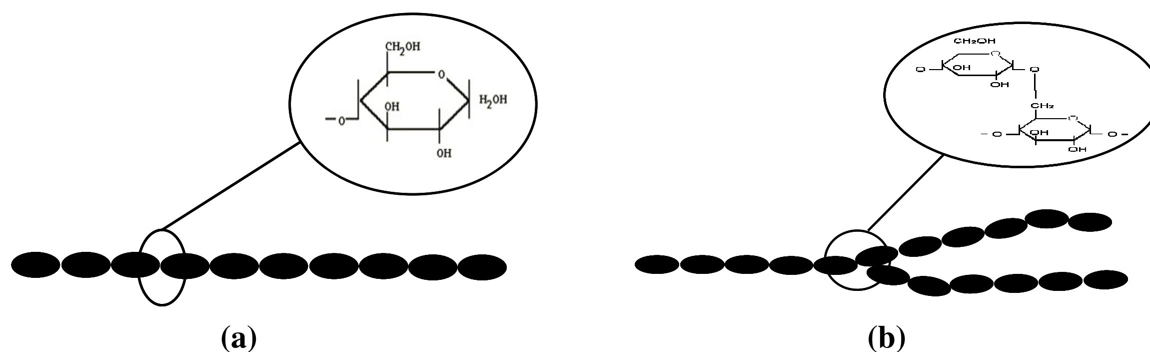

Figure 8 The structure of starch (a) amylose (b) amylopectin

Source: Imeson (1992)

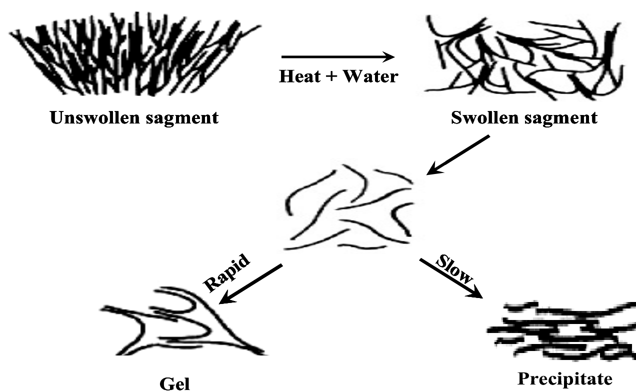

Figure 9 The gel formation of starch

Source: Glicksman (1969)

The starch has been modified to maximize the utilization. Among the modified starch, hydroxypropyl starch is popular, particularly for frozen products due to its stability. It is obtained by an alkaline - catalyzed reaction of starch with propylene oxide. Reaction initially occurs at C-2 and with the low degree of substitution. At higher degrees of substitution, C-3 and C-4 centres are involved (Figure 10). The reaction is carried out on concentrated aqueous suspensions of starch at below the gelatinization temperature. The introduction of hydroxypropyl groups lowers the gelatinization temperature of starch and the temperature should not exceed 50°C during the reaction. If required, a range of viscosities may be obtained by acid hydrolysis of the starch chain as the ether linkage of the substituent groups is not cleaved by acid (Trimble, 1989).

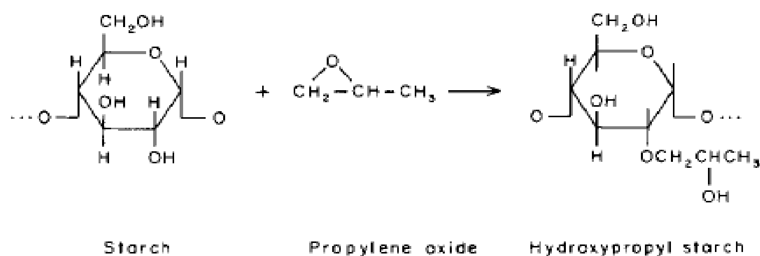

Figure 10 Formation of hydroxypropyl starch.

Source: Trimble (1989)

8.1.2 Application of modified starch

The freezing process induces muscle tissue changes by the formation and accretion of ice crystals, dehydration, and increases in solute (Shenouda, 1980). Freezing and thawing disrupt muscle cells, causing the release of enzymes from mitochondria into the sarcoplasm (Hamm, 1979). Fiber shrinkage and exudation of fluid (drip loss) are deleterious changes in muscle tissue following freezing and thawing (Hale and Waters, 1981). Such an increase in toughness during frozen storage of shrimp and other seafood is attributed to myosin denaturation, as well as cross linking and aggregation of myofibrillar proteins (Sikorski *et al*., 1976). Freeze-thaw processes can also promote lipid oxidation which may affect texture of crustacean muscle (Srinivasan *et al*., 1997). Ramirez *et al.* (2000) suggested that frozen storage of myosin in suspension results in aggregation involving side-to-side interactions of the rod with low formation of disulfide bonds. When myosin is solubilized prior to frozen storage, mainly head-to-head interactions with a higher formation of disulfide bonds are implicated in the aggregations.

When starch is added into surimi seafoods, it modifies texture, improves freeze thaw stability in the case of modifies starch, and decreases the product cost (with addition of water) (Wu *et al*., 1985; Lee *et al*., 1992). Use of starch modified for freeze-thaw stability is a common practice in manufacturing surimi-based products. Stabililized modified starch are commercially prepared by hydroxypropylation or acetylation with or without cross linking so that they resist retrogradation (molecular association) during freezing and thawing, unlike most unmodified starch which undergoes retrogradation and freeze syneresis (water separation) (Lee *et al*., 1992). The chemical groups introduced in the modification disrupt the linearity of the starch molecules, thus interfering with the tendency of the molecules to associate. Syneresis takes places when the starch molecule chains, especially amylose, become tightly associated, resulting in the release of free water from the protein gel. Potato starch readily retrogrades during frozen storage. When such a starch is used in frozen surimi seafood products, the texture becomes hard and rubbery as a result of excessive freeze drip. (Lee *et al*., 1992). The difference in the hydrodynamic behavior between unmodified starch and modified starch is diagramed in Figure 11. (Lee and Chung, 1990). Kim and Lee (1987) reported that the greatest increases in expressible moisture and penetration force were observed in the surimi gels prepared with high-amylose containing starch due to a greater degree of retrogradation. Starch with a high viscosity and good

water-holding ability will produce firm and cohesive surimi gels with the exception of 100% amylopectin and pregelatinized starch. Yang and Park (1998) found that the influences of starch on texture of surimi starch gel depended on the concentration and modification of starch as well as the ratio of amylose and amylopectin. Starch increased the gel strength of surimi-starch gel more effectively at low concentration (30 g/kg) (Yang and Park, 1998). Modification, which facilitated starch granule to swell easily, could increase gel strength (Yang and Park, 1998). The amylopectin component made the granule swell and greatly increased gel strength, whereas higher amylose decrease gel strength (Yang and Park, 1998).

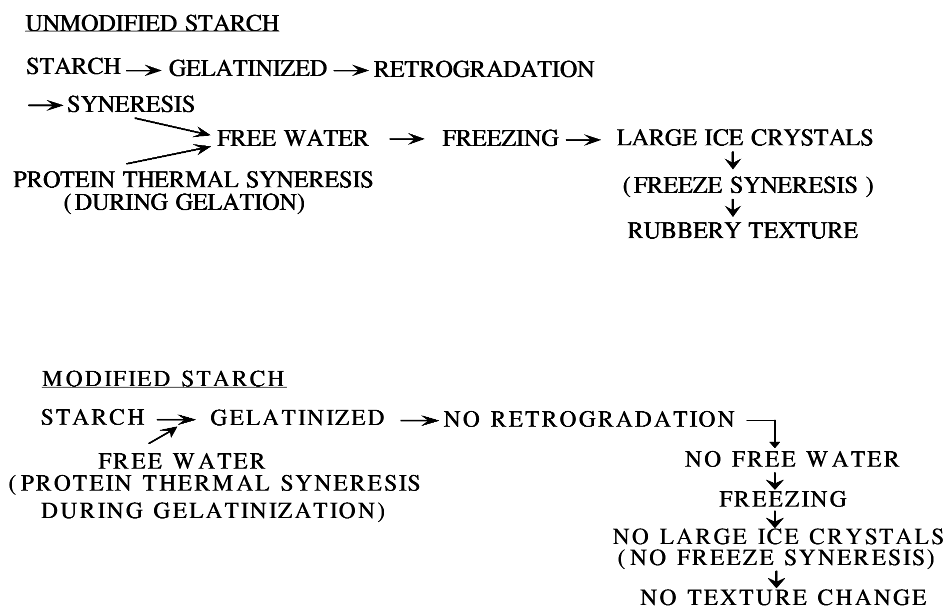

Figure 11 A proposed mechanism of the freeze-thaw stabilizing effect of unretrogradable modified starch

Source: Lee and Chang (1990)

8.2 Carrageenan

8.2.1 The structure of carrageenan

Carrageenan are linear chains of D-galactopyranosyl units joined with alternating (1->3) -α-D- and (1->4)-β-D-glycosidic linkages, with most sugar units having one or two sulfate groups esterified to a hydroxyl group at carbon atoms C-2 or C-6. This gives a sulfate content ranging from 15 to 40%. Unit often contain a 3,6-anhydro ring. The principle

structures are termed kappa (Κ), iota (λ) and lambda (γ) (Figure 12) (BeMiller and Whistler, 1996)

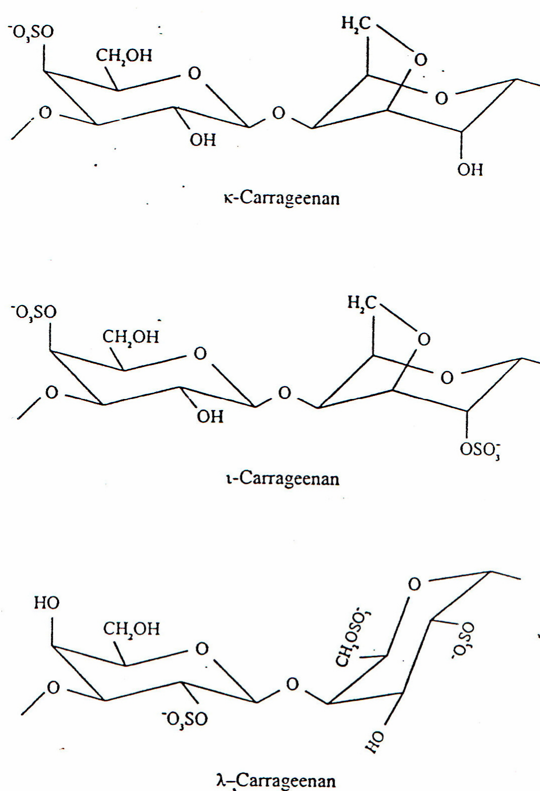

Figure 12 Structures of kappa-, iota-, and lambda-type carrageenans

Source: BeMiller and Whistler (1996)

8.2.2 Functional properties and the application of carrageenan

l-carrageenan consists of alternating 1,3 galactose-4-sulfate and 1,4-linked 3,6-anhydro-D-galactose-2-sulfate and forms elastic gels with calcium ion by a double helix structure. The gels are composed of a spiral of two adjacent molecules held together by ionic and hydrogen bonds which connect galactose oxygens (2 position) on one chain with galactose oxygens (2 position) on another chain. Kappa carrageenan is composed of alternate links of 1,3-galactose sulfate and 1,4 linked 3,6-anhydro-D-galactose. It is characterized by its gelling ability via potassium ion and forms brittle gels without (or elastic gel with) the aid of locust bean gum. Both iota and kappa carrageenan form gels via double helix interaction (Figures 13 and 14). In general, double helices are neither common nor necessary in gelation of polysaccharide (Arnnott, 1977).

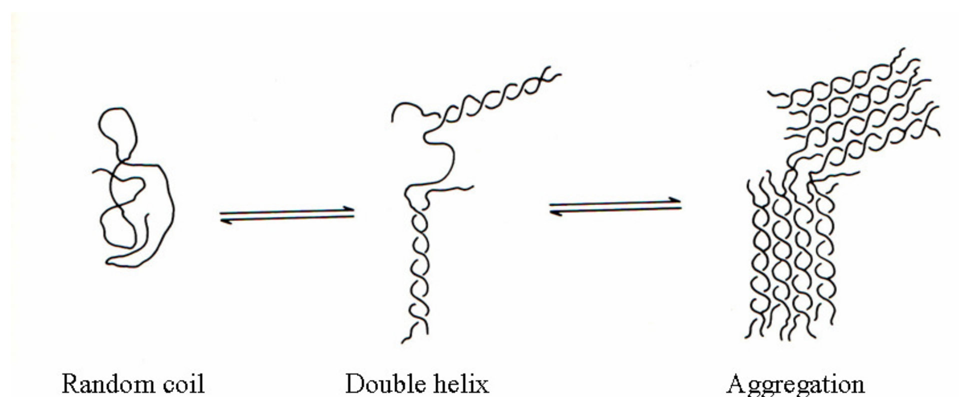

Figure 13 Gelation of kappa and iota carrageenan

Source: Robin *et al* (1975)

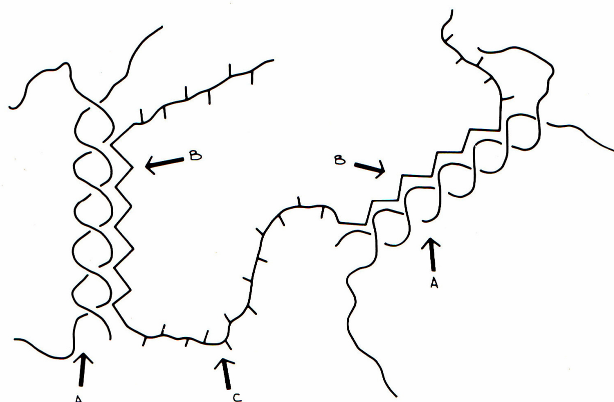

Figure 14 Cross-linking helical kappa carrageenan; (A) by binding of 'smooth' regions of locust bean gum; (B) the parts of the galactomannan which carry grouped galactose substituents and which we term 'hairy' region; (C) form flexible connections between helices.

Source: Rees (1972)

8.2.3 Application

Iota-carrageenan markly increased gel strength by synergistic compounds such as Ca^{2+}-bridge-forming agent (Bullens *et al*., 1990; Lee and Chung, 1990)**.** Carrageenan can be used in the analog formulation from 0.1 to 0.5%. The surimi gel strength can be significantly improved when carrageenan was used at level of 0.25% together with whey protein concentrate

(Bullens *et al*, 1990). Guillin, *et al* (1997) reported that addition of starch gave less rigidity than iota-carrageenan, although it stabilized the formed gel at high temperatures (80°C). Iota-carrageenan (Llanto *et al*., 1990) and kappa-carrageenan (Niwa, 1992) bind the water in the system. Carrageenan can also combine with other gums, such as konjac (glucomannan) and locust (carob) bean gum to improve the strength, elastic, and / or water-holding ability of the gel (Lee *et al*., 1992). Foegeding and Ramsey (1987) found that iota - carrageenan was most effective at increasing water holding ability of gelled meat batter, compared with keppa-carrageenan and xanthan gum. Different carrageenans had different effect on meat batters. Lambda-carrageenan could increase water-holding capacity of meat batter, but it could decrease gel strength of cooked products (Foegeding and Ramsey, 1987). Kappa-carrageenan could increase gel hardness of non-fat and non-water added emulsified meat products (lipid content 4%) (Trius *et al*., 1994). Hsu and Chang (2001) reported that the addition of kappa-carrageenan at level less than 2% significantly affected the product cooking yield, hardness, adhesion, chewiness, gumminess and viscosity of low fat emulsified meat balls. Goa *et al* (1999) reported that carrageenan increased water retention and improved gelling ability of mince fish by interaction with fish myofibrillar protein.

OBJECTIVES

1. To study the effect of sodium chloride and neutralized phosphate compounds in combination with magnesium chloride on gel forming ability of Pacific white shrimp meat.
2. To investigate the autolysis of Pacific white shrimp meat and to study the effects of some protein additives on autolysis inhibition and gelling properties of resulting gel.
3. To study the effect of setting condition on gel forming ability of Pacific white shrimp meat and to elucidate the efficiency of MTGase on the quality improvement of gel produced from Pacific white shrimp meat.
4. To investigate the effect of modified starch and ι-carrageenan on freeze-thaw stability of Pacific white shrimp gel.