Chapter 1

Introduction

Surimi is the minced fish flesh, washed to remove most of lipids, blood, enzymes and sarcoplasmic proteins and stabilized for frozen storage by cryoprotectants. The myofibrillar protein in the resulting product is high in myofibrillar proteins, which possess gel forming ability (Benjakul *et al*., 2003). Generally, gel strengthening of surimi can be achieved by subjecting surimi sol to setting below 40 °C prior to cooking (An *et al*., 1996; Kimura *et al*., 1991). Gelation of fish paste during setting has been reported to have a close relationship to the formation of cross-linking between myosin heavy chain induced by endogenous transglutaminase (TGase) (Kumazawa *et al*., 1995; Seki *et al*., 1990) as well as to the thermal formation of non-covalent bonds and disulfide bonds (Hossain *et al*., 1998). Benjakul *et al*. (2001) reported that natural actomyosin from bigeye snapper underwent aggregation at temperatures above 30°C. Formation of large aggregates is presumably a prerequisite to formation of a good elastic gel (Chan *et al*., 1992). High temperatures during heating led to further oxidation of sulfydryl groups with a subsequent disulfide bond formation (Benjakul *et al*., 2001).

Phosphates have been widely accepted as additives in fish and seafood because they improve functional properties during processing by increasing water retention in fresh products and reducing the thaw loss in frozen fish (Chang and Regenstein, 1997b). Phosphate is commonly added to surimi in combination with cryoprotectants such as sugar or sorbitol. It functions as a metal chelator and/or

antioxidant. The raising pH caused by this compound results in the improved water holding/binding of the gel as well as better salt solubilization of myofibrillar proteins (Park, 2000). Pyrophosphate was reported to dissociate protein complex, leading to the improved gel forming ability (Matsukawa *et al*., 1995). Although phosphate compounds have been proven as the promising processing aid, it probably shows the detrimental effect on gel property since it could chelate the Ca^{2+}-ion. This may impede the setting of surimi induced by endogenous TGase. Nevertheless, the information regarding the effect of phosphate compounds on gelling property of surimi from tropical fish is scarce. Therefore, the use of phosphates with the appropriate type and level in combination with the sufficient amount of calcium ion or cross-linking enzymes would be a promising means in improving the quality of surimi gels.

Literature Review

Fish muscle is a major source of proteins possessing the various functionalities including gelation. Myofibrillar proteins in white muscle of fish represent 65-75% of total proteins (Mackie, 1994) and are the largest fraction of proteins of muscle tissue. They influence the meat's culinary and functional properties due to their high water binding capacity and emulsifying capacity. Properties and possible implications of myofibrillar proteins are related with meat quality (Pomeranz, 1991). The interactive properties of myosin and actin are of particular importance in determining functional properties of protein from fish, especially gel-forming ability (Mackie, 1994).

The conformation, shape and size of the protein molecules have remarkable effects on their functionalities. Protein functionality in meat products is generally induced by major alterations in the native structure of the molecules, which are typically accomplished by manipulation of heating and cooling procedures (Xiong, 1997).

The main constituents of fresh fish are water (65-85%), protein (15-24%), fat (0.1-22%), carbohydrate (1-3%) and inorganic substances (0.8-2%). The amount of fish meat varies according to the species, age, part of body, pre- or post-spawning season and the feeding condition (Suzuki, 1981). Protein is a major composition of fish muscle with the range of 15-20% (wet weight). Protein compositions of fish vary, depending upon muscle type, feeding period, and spawning, etc. Generally, protein content is reduced in spawning period (Almas, 1981).

Muscle protein compositions

Protein composition in the muscle can be divided into 3 main groups as follows:

1. Sarcoplasmic proteins

Sarcoplasmic proteins comprise about 20-35% of the total muscle proteins and are commonly called myogens, which include most water-soluble proteins (Mackie, 1994; Pearsons and Young, 1989). Sarcoplasmic proteins occupy about 25% of the cellular space and have a concentration of about 260 mg/ml (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. These structural characteristics may be partially responsible for the high solubility of these proteins in water or dilute salt solutions (Ochiai and Chow, 2000).

2. Stroma proteins

Stroma is the protein, which forms connective tissue, representing approximately 3% of total protein content of fish muscle. It cannot be extracted by water, acid, or alkali solution and neutral salt solution of 0.01-0.1 M concentration. The stroma involves collagen, elastin or both (Suzuki, 1981). Elastin is very resistant to moist heat and cooking. Normally, the different structural arrangements of muscle cells in fish are observed, compared to mammals (Mackie, 1994).

3. Myofibrillar proteins

Myofibrillar proteins are the major proteins in fish muscle. These

proteins account for 65-75% of total protein in muscle, compared with 52-56% in mammals (Mackie, 1994). Myofibrillar protein can be further divided into three subgroups as follows:

- **Myosin**

Myosin is the protein, which forms the thick filament. A molecular weight is about 500,000 Daltons. It is the most abundant myofibrillar component, constituting approximately 40-60% of total protein content. Myosin contains a total of six polypeptide chains, two heavy chains and four light chains. The six polypeptide chains of myosin are assembled in a quaternary structure that resembles a stick (tail) with two pear-shaped heads (Figure 1) (Foegeding *et al*., 1996; McCormick, 1994). The tail regions consists of two alpha-helical heavy chains coiled together into a coiled-coil alpha-helical supersecondary structure. This structure terminates at the head region. The main secondary structure in the head is alpha-helix, accounting for approximately 48% of amino acids. The myosin head contains the actin binding site, ATPase 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).

Myosin ATPase activity is stimulated by Ca^{2+}. 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).

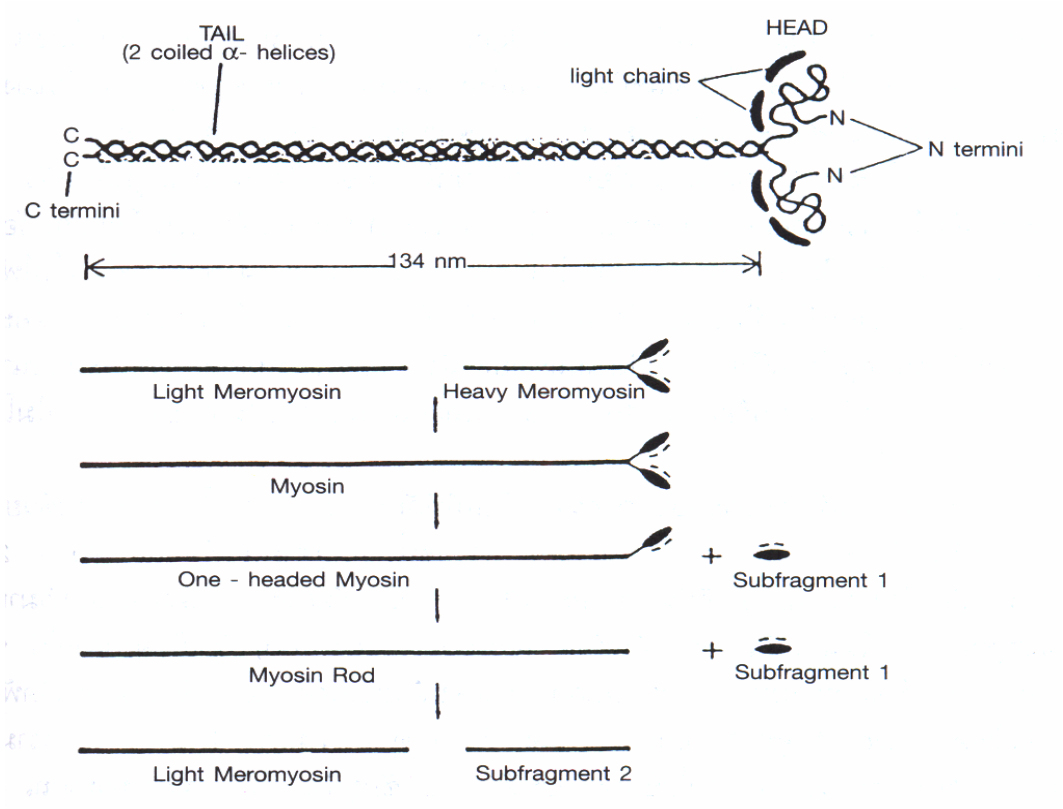


Figure 1. Structure of myosin.
Source: McCormick (1994)

- Actin

The major protein of the thin filaments is actin, which comprises 20% of myofibrillar proteins of muscle. Actin consists of two peanut shaped domains of equal size lying side-by-side. Actin monomers, called globular actin or G-actin, are assembled in a double-helical structure called fibrous actin, or F-actin (Figure 2). G-actin has a molecular mass of 42,000-48,000 Daltons. It is stable in water, where it can also exist as a dimmer (Xiong, 1997). Globular actin binds ATP very firmly and, in the presence of Mg^{2+}, spontaneously polymerizes to form F-actin. It also polymerizes in the presence of neutral salts at a concentration of approximately 0.15

M. Filaments of F-actin interact with the head portion of myosin (Foegeding *et al*., 1996).

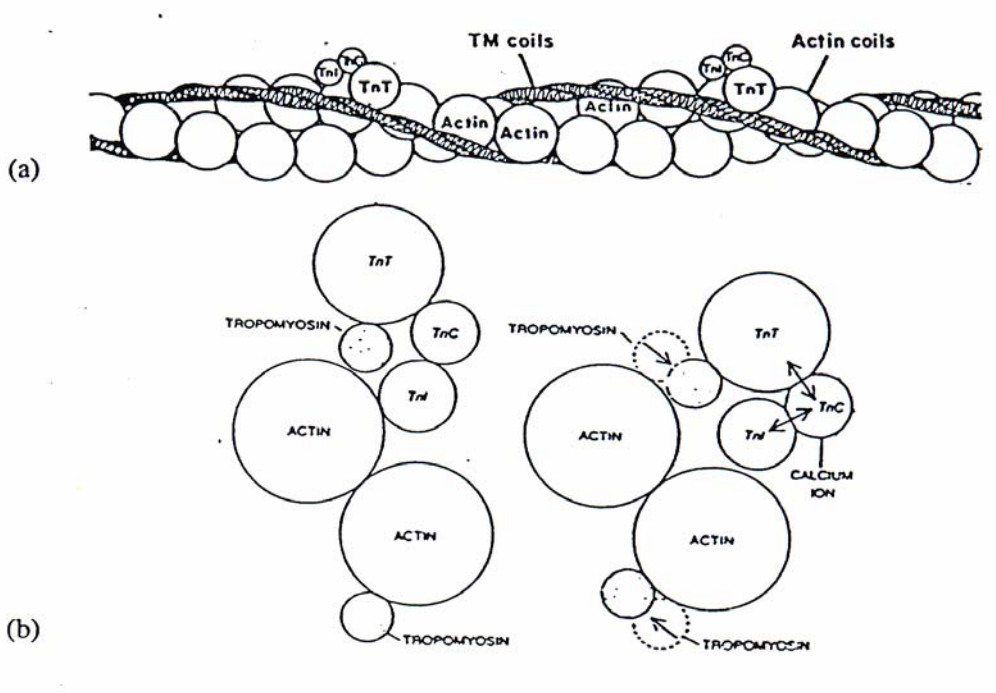


Figure 2. a) Proteins of the thin filament
b) Arrangement of actin, troponin and tropomyosin.
Source: McCormick (1994)

- **Actomyosin**

When actin and myosin are mixed *in vitro*, a complex, called actomyosin, 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 (Ochiai and Chow, 2000). However, unlike prerigor muscle, simple addition of ATP and other solubilizing compounds such as Mg^{2+} to meat does not dissociate all the myosin from actin, and extraction of myosin from postrigor meat is therefore difficult (Foegeding *et al*., 1996). Myosin and actomyosin are generally found in extracts of postmortem muscle (Foegeding *et al*., 1996). The dissociation constant for actin and myosin is 10^{-8} to 10^{-7} M. The

actomyosin specifically dissociates with ATP, pyrophosphate, and other polyanions. The higher the ionic strength, the less ATP is required. Mg^{2+} is also required for the dissociation (Ochiai and Chow, 2000).

- **Troponin**

Troponin accounting for 8-10% of myofibrillar proteins consists of three subunits designated troponin C (for calcium binding), troponin I (for inhibitory), and troponin T (for binding with tropomyosin). Each subunit of troponin has distinct functions. Troponin C is a calcium binding protein and confers calcium regulation to the contractile process via the thin filament (Figure 2) (Foegeding *et al*., 1996).

- **Tropomyosin**

Tropomyosin represents approximately 8-10% of myofibrillar protein. It is composed of two alpha-helical polypeptides wound together into a two-stranded, coiled-coil supersecondary structure. It resembles the tail or rod portion of the myosin molecule. In skeletal muscle, two polypeptides, alpha- and beta-tropomyosin polypeptides have molecular masses of 37,000 and 33,000 Daltons, respectively. Tropomyosin aggregates end-to-end and binds to actin filaments along each groove of the actin double helix such that each molecule interacts with seven G-actin monomer (Figure 2) (Foegeding *et al*., 1996).

4. Surimi and surimi production

Surimi is minced fish extracted with water to remove strong flavor, pigments, and nonfunctional proteins and subsequently dewatered to concentrate the myofibrillar proteins. The flesh is separated from bones and skin (usually

mechanically) and is called “minced fish”. The mince is used as the starting material for surimi production and as ingredient for some processed fish products, such as fish sticks, fish cakes, etc. The removal of water-soluble proteins, fat, connective tissues and undesirable muscle components such as blood and pigments is needed for surimi production (Suzuki, 1981). These constituents are thought to interfere with gel formation (Lee, 1984; Hultin, 1985). Washing also functions to concentrate the desirable myofibrillar proteins, particularly myosin (Kudo *et al*., 1973). Myosin has been known as the predominant protein involved in gelation (Itawa *et al*., 1977; Shimizu *et al*., 1983; Akahane *et al*., 1984; Sano *et al*., 1989). Myofibrillar protein will lost their functional properties rapidly when they are frozen. Therefore, the raw surimi is generally mixed with cryoprotectant such as sucrose, sorbitol, and polyphosphate (Park and Morrissey, 2000). The use of fresh fish for the surimi production is also essential since tissue autolysis by endogenous proteolytic enzymes during storage reduces the level of extractable actomyosin available for gelation (Makinodan *et al*., 1980; Kim *et al*., 1982; Benjakul *et al*., 2002; Benjakul *et al*., 2003).

The addition of cryoprotectants is important to ensure maximum functionality of frozen surimi because freezing induces protein denaturation and aggregation (Benjakul and Bauer, 2000). Sucrose and sorbitol, alone or mixed at about 8% (w/w) are commonly added to dewatered fish meat, serve as the primary cryoprotectants in the manufacture of surimi. Sucrose is cryoprotectant commonly used in surimi. The cryoprotective role of sucrose is to prevent actomyosin denaturation during frozen storage (Turner *et al*., 2001). Sorbitol has been used to reduce the level of sucrose in surimi. In addition, a mixture (1:1) of sodium tripolyphosphate and tetrasodium pyrophosphate at 0.2-0.3% is commonly used as a

synergist to the cryoprotective effects of carbohydrate additives (Park and Morrissey, 2000). Extended frozen storage of surimi is made possible by the incorporation of sucrose and sorbitol which have been shown to be effective in inhibiting fish protein denaturation (Sych *et al*., 1990) and by polyphosphates which also have been shown to induce stabilization of myosin (Park and Lanier, 1987; Noguchi and Matsumoto, 1971). The addition of cryoprotectants (12% w/w sucrose and 0.2% w/w polyphosphates) to fresh Pacific whiting stabilized fish muscle proteins and maintained textural quality during frozen storage (Simpson *et al*., 1994).

Process and additives required for surimi vary with fish used. Different fish have the varying intrinsic enzymes and proteins associated with gelation. Enzyme inhibitors are required for some surimi facing with gel softening unless the surimi is cooked rapidly using an ohmic heater (Yongsawatdigul *et al*., 1995) or is thinly extruded and cooked rapidly, like in crabstick processing (Park and Morrissey, 2000).

Pacific whiting and arrowtooth flounder (*Atheresthes stomias*) are the fish that require enzyme inhibitors to minimize textural deterioration caused by a heat-stable enzyme (Greene and Babbitt, 1990). Gel weakening at around 55-60ºC has also been reported in threadfin bream (*Nemipterus bathybius*) (Toyohara and Shimizu, 1988), Atlantic menhaden (*Brevoorti tyrannus*) (Boye and Lanier, 1988), white croaker (*Micropogon opercularis*), and oval filefish (*Navodon modestus*) (Toyohara *et al*., 1990).

To make surimi from oily/dark or red-fleshed fish, such as mackerel, sardine, and salmon, certain steps must be applied to negate the effects of the oil and heme proteins. Heme proteins, such as myoglobin and hemoglobin, account for the red color of dark muscle. In addition, fat oxidation in the dark muscle is promoted by heme proteins, which causes an offensive and rancid odor (Tokunaga and Nishioka,

1988). Addition of 0.1-0.5% $NaHCO_3$ in the first washing solution and a further decanter is commonly used to remove the extra oil. The addition of 0.05-0.1% sodium pyrophosphate and the use of vacuum during washing are also recommended to remove heme proteins.

5. Gelation of muscle proteins

Gelation is one of important functionalities of muscle proteins. Gelling property mostly depends on the muscle compositions as well as the gelation process, such as thermal induced gelation, acid induces gelation, etc. Gelation of myofibrillar proteins has been shown to be largely responsible for the textural properties of processed fish product (Xiong and Brekke, 1989). 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, 1979). Gel-forming ability of frozen surimi is the most important functional requirement of imposing good quality of surimi-based products (Saeki *et al*., 1995). Differences in cross-linking of MHC contribute to the differences in gel-forming ability among the muscles of various fish (Benjakul *et al*., 2001). Protein gels are three-dimensional matrixes or network in which water is entrapped (Pomeranz, 1991).

The process of protein gelation involves two steps (Ziegler and Aton, 1984) as follows:

1. Protein denaturation

Addition of salt in combination of heating are two major factors involved in denaturation and gelation of muscle proteins. Table 1 gives a summary of changes, which may occur during the heat denaturation of actomyosin.

Table 1. Conformational changes which may occur during the thermal denaturation of natural actomyosin

Temperature °C	Protein (s) or segment involved	Description of events
30-35	Native tropomyosin	Thermally dissociated from the F-actin backbone
38	F-actin	Super helix dissociates into single chains
40-45	Myosin	Dissociates 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. Aggregation

Denatured proteins begin to interact 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, exposing reactive surfaces of neighboring protein molecules, which then interact to form intermolecular bonds (Lanier, 2000). The number and kind of interactions or bonds are not only affected by the species from which the surimi is derived (Suzuki, 1981; Shimizu, 1985) but also the heat conditions in which the gel is made (Ishikawa, 1978; Akahane and Shimizu, 1990; Lee *et al*., 1990; Yamazawa, 1990). Chan *et al*. (1992) 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 of proteins.

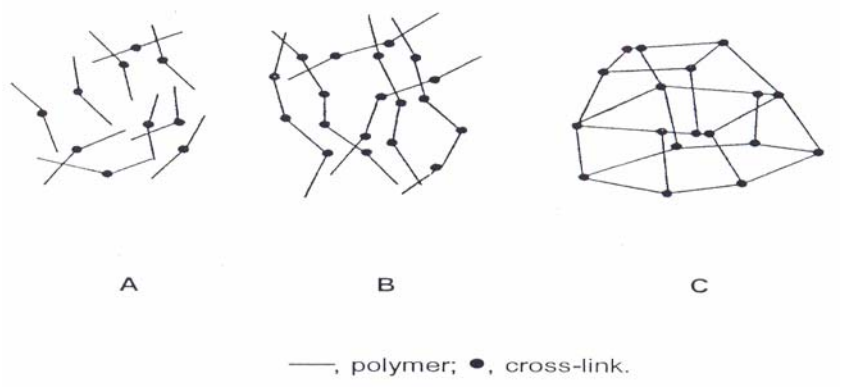


Figure 3. Formation of a gel network structure
Source: Niwa (1992)

Chan *et al*. (1994) compared the denaturation and aggregation behaviors of cod and herring myosins and reported that the inferior gel forming ability of herring muscle proteins was related to the poor unfolding profile of interior hydrophobic domains when heated. Extent of aggregation for fish myosin seems to depend on the amount of hydrophobic surface exposed on the heated molecules (Chan *et al*., 1992; Wicker *et al*., 1986). Therefore, the extent of aggregation for poor gel forming species, i.e. herring, would be improved by finding a strategy to increase the surface hydrophobicity of heated myosins. Gill *et al*. (1992) reported that myosins from different fish species aggregated to different extents as the temperature increased. The head portions associate to form "super-junctions" which provide extra cross-linking to the gel network. Since agents that block formation of disulfide bonds did not prevent the aggregation of the myosin head but only retarded its onset, Semejima *et al*. (1981) concluded that another type of aggregation, perhaps due to

intermolecular association of side chains, superimposes on the sulfhydryl-dependent reaction. Covalent bonds such as disulfide bonds are dominant when heated at high temperatures (>40°C) (Lanier, 2000). Heating actomyosin solution at 40 and 85°C resulted in reduction of ATPase activity. Sano *et al*. (1994) found that reactive SH increased from 20 to 50°C, suggesting that SH groups inside the actomyosin molecule emerged to the surface as a results of unfolding, thereby causing a gradual decrease in ATPase activity with the increase in temperature. A rapid loss in enzyme activity was found from 40 to 50°C, indicating conformational changes in active sites in actomyosin.

Hydrogen bonds are weaker dipole bonds not responsible for the gelation of myofibrillar proteins but are important in the stabilization of bound water within the hydrogel. Calcium ions can form salt linkages between negatively charge localized on two adjacent proteins (Wan *et al*., 1994). Though the addition of calcium ions may contribute to the strengthening of surimi gels, but this type of bond will not cause gelation of surimi in itself. The addition of calcium salts to improve gelling properties of surimi may actually be more due to their effects on a crosslinking enzyme, transglutaminase, in the muscle than from ionic linkages between proteins.

For surimi gelation, rigidity of the previously formed elastic network caused by setting is enhanced as aggregation continues, particularly at above 60-70°C (Roussel and Cheftel, 1990; Sano *et al*., 1990). This can be attributed to a network formation of the fibrous myofibrillar protein molecules. The configuration of the protein is changed by heat with an interaction of a radical group on the molecular surface, forming a network far stronger than that of suwari gel. The tail region predominantly involves in cross-linking at lower temperatures, while the globular

head portion (HMM S-1) of myosin takes action above 60-70°C (Liu *et al*., 1982; Taguchi *et al*., 1987; Sano *et al*., 1990).

Sano *et al*. (1990) studied the dynamic viscoelastic behaviors and turbidity of isolated carp HMM and LMM and concluded that the initial development of gel elasticity was attributable mainly to the LMM and a second stage was due to HMM. Sano *et al*. (1990) proposed that the bulk of the protein-protein interactions over the range 50-80°C were those of the head-head type. On heating actomyosin solutions from 53 to 80°C, Sano *et al*. (1989) noted that the rate of elasticity development increased with F-actin content. This was attributed to the dissociation of actin filaments (Sano *et al*., 1989). However, F-actin alone formed a viscous and curdy sol when exposed to the same thermal treatment. Gill and Conway (1989) also showed that the tail of the myosin molecule rather than the head was involved in thermal aggregation. On the other hand, Taguchi *et al*. (1987) reported that the extent of aggregation for S1 was higher than HMM and LMM at the initial stage. Both HMM and LMM are involved in thermal aggregation of cod and herring myosins. 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 aggregates at higher temperatures (Figure 4) (Chan *et al*., 1993).

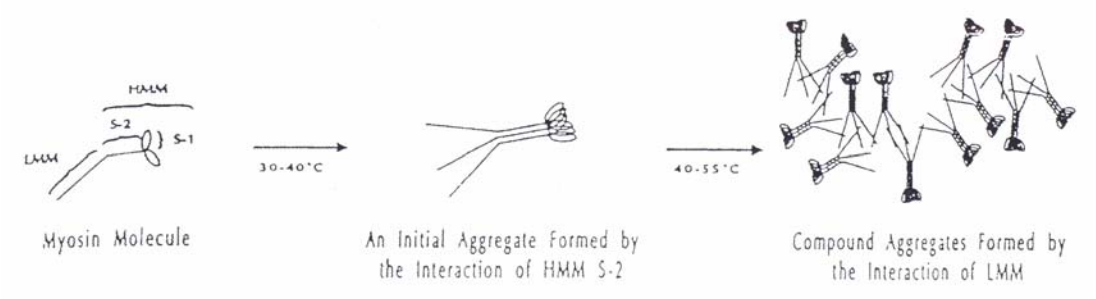


Figure 4. Schematic representation of the thermal aggregation of fish myosin.
Source: Chan *et al.* (1993)

6. Role of endogenous transglutaminase in setting and protein cross-linking

TGase is a transferase, having the systematic name as protein glutamine γ-glutamyltransferase (EC 2.3.2.13). It catalyzes the acyl transfer reaction between γ-carboxyamide groups of glutamine residues in proteins peptides, and various primary amines. When the ε-amino group of lysine acts as acyl acceptor, it results in inter- or intra-molecular cross-linking of protein via formation of ε-(γ-glutamyl) lysine linkages. This occurs through exchange of the ε-amino group of the lysine residue for ammonia at the carboxyamide group of a glutamine residue in the protein molecule(s) (Figure 5). In the absence of primary amines, water may act as the acyl acceptor, resulting in deamination of γ-carboxyamide groups of glutamine to form glutamic acid. Formation of covalent cross-links between protein is the basis of the ability of TGase to modify the physical properties of protein foods (Ashie and Lanier, 2000). Tsukamasa and Shimizu (1990) also detected TGase activity in fish muscle including sardine, Pacific mackerel, red sea bream, horse mackerel, ayu, carp silver eel and Japanese Spanish mackerel. Araki and Seki (1993) estimated that the ratio of TGase unit to actomyosin in fish muscle was roughly 0.093-0.004 unit TGase to 5 mg actomyosin. Bejakul and Visessanguan (2003) reported the role of TGase in setting of bigeye snapper surimi. Optimum pH and temperature of TGase activity from bigeye snapper (*P. tayenus*) were 6 and 40°C, respectively (Benjakul and Visessanguan, 2003).

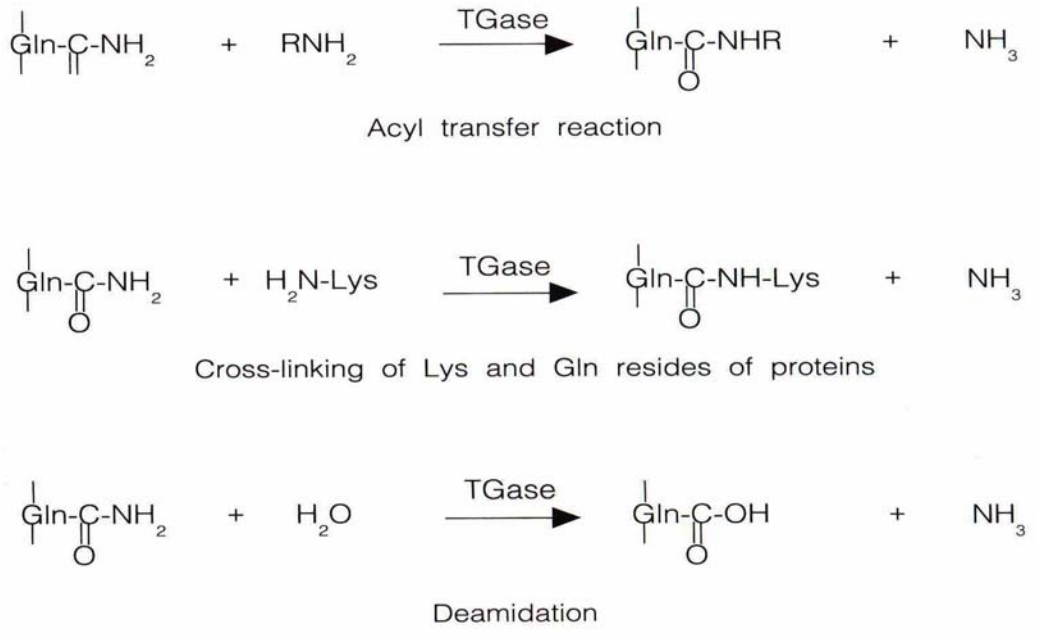


Figure 5. Reaction catalyzed by TGase
Source: Ashie and Lanier (2000)

Endogenous TGase is water soluble and can be removed if washing is too extensive (Nowsad *et al*., 1994a,b). TGase content can vary greatly with the type and extent of process used during surimi manufacture. Nowsad *et al*. (1995) showed that the sarcoplasmic fraction of fish can actually enhance the gelling ability when added back to surimi because of its higher TGase activity. It is likely that different fish species, and perhaps different individuals within species, could vary in natural content of the enzyme, possible affected by habitat, feed and physiological condition. α_2-macroglobulin component of fish blood plasma (or added beef plasma) is postulated to has the ability to form ε-(γ-glutamyl) lysine cross-links in fish protein (Kang and Lanier, 1999). It has been shown that in certain species, such as salmon, the water-soluble fraction of muscle also contains factors that inhibit TGase activity (Wan *et al*., 1994).

Setting or suwari is a well known occurrence in the surimi paste during the incubation at temperatures lower that 40°C. Setting phenomenon caused by endogenous TGase may be varied between species and contributes to the different gelation characteristics. Benjakul and Visessanguan (2003) found that setting of surimi paste with and without subsequent heating resulted in the increase in both breaking force and deformation of suwari and kamaboko gel from bigeye snapper surimi. Suwari and kamaboko gels with the setting at 25°C had a lower solubility, when the setting time increased. The decrease in solubility indicated the formation of non-disulfide cross-links induced by endogenous TGase (Benjakul and Visessanguan, 2003). Seki *et al*. (1990, 1998); Kimura *et al*. (1991); Kamath *et al*. (1992); Wan *et al*. (1994) reported that an endogenous TGase was largely responsible for the setting phenomenon. Wan *et al*. (1995) confirmed the essential role of TGase in the setting of walleye pollack surimi paste, compared with that of salmon, non-setting fish. The TGase is calcium-dependent enzyme that catalyzes the cross-linking of certain glutamine and lysine side chains in myosin heavy chains in salted surimi paste during setting prior to cooking. In easy setting fish species, the thermal gelation of myosin is modified to various extents by the formation of covalent cross-links, depending on the enzymatic activity in the surimi paste during the setting.

The amount of dansyl-glutamine incorporated into muscle protein increased with the prolongation of incubation in the fish flesh sols. Large increment was found in Alaska pollack and Pacific mackerel but not so large in horse mackerel (Hossain *et al*., 1998). The increase in gel strength of surimi from Alaska pollack was associated with the increased cross-linking of MHC and ε-(γ-glutamyl)lysine content formed (Kumazawa *et al*., 1995). Conversely, inhibition of endogenous TGase

resulted in complete suppression of myosin cross-linking of walleye pollack surimi gel and the cross-linking was also inhibited above 45°C due to the inactivation of TGase (Takeda and Seki, 1996). The ε-(γ-glutamyl)lysine formation in Alaska pollack gel was suppressed by addition of EDTA and ammonium chloride (Kumazawa *et al*., 1995).

Gelling properties of surimi are determined by level of endogenous TGase and the conformation of protein substrates. Wan *et al*. (1995) found that the poorer gel-forming ability of salmon was due to the lower TGase activity as well as the lower contents of myosin and calcium ion, compared with walleye pollack surimi. Moerover, the reactivity of TGase to various fish actomyosin was markedly different (Araki and Seki, 1993) and depended on the conformation of actomyosin. Fish TGase is Ca^{2+}-dependent, however, the requirement of Ca^{2+} ion varies among fish species (Nozawa *et al*., 1997). The addition of calcium compounds to surimi enhanced TGase –mediated setting, resulting in stronger gels (Lee and Park, 1998).

7. Improvement of gel quality of surimi

Gelation of fish proteins is the most important step in forming desired textures in many seafood products, particularly those from surimi. Various physical conditions and chemical additives can affect surimi gelling property. Enzyme inhibitors, such as beef plasma protein, egg whites, whey protein concentrates, or potato extracts, have been used in conjunction with cryoprotectants, gel enhancers, and color enhancers (Park and Morrissey, 1994; 2000). Sucrose, sorbitol, sodium tripolyphosphate, tetrasodium pyrophosphate, calcium compounds (calcium lactate, calcium sulfate, calcium citrate, calcium caseinate), sodium bicarbonate,

monoglyceride or diglyceride, and partially hydrogenated canola oil can be added into surimi (Park and Morrissey, 2000). The addition of enzyme inhibitors or calcium compounds before freezing surimi is not necessary, especially because added calcium compounds can actually enhance protein denaturation during frozen storage. Instead, these compounds can be added when the surimi paste is prepared to make gels (Lee and Park, 1998). Additionally, microbial TGase has been widely used to increase the gel strength of surimi (Seki, *et al*., 1990; Ni, *et al*., 1998; Wang and Lanier, 1998; Soeda, *et al*., 1996).

Use of sodium chloride

The addition of salt shifted the denaturation transitions to lower temperatures and decreased the enthalpies of heat denaturation. 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 aggregation of cod myosin at heating temperatures higher than 50°C. Alvarez *et al*. (1995) studied the effects of salt concentration on sardine surimi gels. Maximal gel strength was obtained at salt concentrations of 2.24% NaCl. The formation of a firm and elastic kamaboko gel from sardine surimi requires the addition of 2-4% NaCl (Roussel and Cheftel, 1990). Chen (1995) found that the breaking force of surimi from shark muscle increased with increasing NaCl concentration, but deformation increased at lower NaCl concentration and then decreased when NaCl concentration reached 1.6 M.

Use of calcium ion

Calcium compounds are commonly added as surimi gel enhancer. Calcium ion must be present for the endogenous TGase to active and induce setting (Lee and Park, 1998). Yamamoto *et al*. (1991) patented the use of a mixture of sodium bicarbonate, calcium citrate, and calcium lactate as gel quality improving agents. Yasuyuki *et al*. (1993) reported that ε-(γ-glutamyl)-lysine crosslink content and breaking force of gels from sardine surimi increased when $CaCl_2$ was added in combination with setting at 25°C. Lee and Park (1998) concluded that the textural properties of surimi could be improved maximally with a 25°C preincubation and the addition of 0.1% calcium lactate or 0.05% calcium acetate for Alaska pollock and 0.2% calcium lactate for Pacific whiting, respectively. Benjakul *et al*. (2004c) found that the addition of $CaCl_2$ increased breaking force and deformation of suwari gel of surimi from bigeye snapper, threadfin bream, barracuda and bigeye croaker. The decrease in solubility of suwari gel in mixture of sodium dodecyl sulfate, urea and β-mercaptoethanol suggested non-disulfide covalent bond formation. Ca^{2+}-ion required can be varied with fish species. Walley pollack TGase required 3 mM Ca^{2+}-ion, while carp muscle TGase required 5 mM Ca^{2+}-ion for full activation (Kishi *et al*., 1991). TGase from red sea bream liver and carp muscle required 0.5 mM and 5 mM Ca^{2+} for full activity, respectively. The addition of 1.5% calcium carbonate resulted in the increases in breaking force and deformation of mixed SSA grade surimi from bigeye snapper and mackerel (7:3 ratio) (Benjakul *et al*., 2004b). Walleye pollack surimi pastes having different Ca^{2+} concentrations, incubated at 25°C prior to heating at 90°C, had different cross-linking myosin heavy chains and varying incorporation of

MDC into surimi paste. The maximal cross-linking and MDC incorporation were obtained in the presence of Ca^{2+} in the range of 2-5 mM (Wan *et al*., 1994).

Use of microbial transglutaminase (MTGase)

The commercial development of a low cost source of TGase from microbial culture offers a means of upgrading the gelling quality of surimi (Ando *et al*., 1989). This microbial TGase is not calcium sensitive; therefore, neither chelating agents nor calcium salts have any marked effect on its activity (Lanier, 2000). MTGase from *Streptoverticillium* has a molecular weight of 40 kDa (Ando *et al*., 1989). The Ca^{2+} - independent MTGase from *Streptoverticillium mobarense* (Nonaka *et al*., 1989; Huang *et al*., 1992; Gerber *et al*., 1994) or from *Streptoverticillium ladakanum* (Tsai *et al*., 1996) has shown potential to increase the gel strength of fish surimi. Ando *et al*. (1989) isolated microorganism (*Streptoverticillium mobaraense*) that produced a TGase which did not require calcium ions for activity. MTGase has been applied to polymerize rabbit myosin, carp myosin, beef myosin and actin (Nonaka *et al*., 1989, 1994; Muguruma *et al*., 1990; Kato *et al*., 1991). MTGase has been shown to be useful in strengthening surimi gels during the setting reaction (Seguro *et al*., 1995; Sakamoto *et al*., 1995a). The gel-forming and viscoelastic properties of surimi-based products were improved by addition with MTGase (Seguro *et al*., 1995). Tsukamasa and Shimizu (1990) reported that the strong gel-forming ability of sardine was due to the formation of the non-disulfide bond, which was shown to be due to the action of TGase (Tsukamasa *et al*., 1993).

Addition of microbial TGase to surimi significantly increases its gel strength, particularly when the surimi has lower natural setting ability (presumably due to lower endogenous TGase activity) (Lee *et al*., 1997; Seguro *et al*., 1995;

Kumazawa *et al*., 1993a). An increase in non-disulfide polymerization and formation of ε-(γ-glutamyl) lysine dipeptides was found with increase in setting time and microbial TGase concentration (Tsukamasa and Shimizu, 1990). At equal levels of ε-(γ-glutamyl) lysine content, gels prepared with added MTGase displayed higher gel stress (Lee *et al*., 1997). Since these gels achieved this level of isopeptide content more rapidly than pastes gelled with endogenous TGase only, it was concluded that the rate of myosin polymerization may also be a factor influencing gel strength, not isopeptide content alone. Yasunaga *et al*. (1996) observed that increased isopeptide content was concomitant with increased gel strength and increased amount of microbial TGase added. The relatively higher gel strength with an increasingly lower gel strain was observed, compared with those produced by setting without additives (Abe, 1994).

Crosslinking of myosin heavy chains via ε-(γ-glutamyl)-lysine formation increased when MTGase was added (Seguro *et al*., 1995). Effective concentrations of MTGase on the formation of cross-links was 1.5-3.0 unit/g protein for high grade pollock surimi and 3.0-5.0 unit/g protein for low grade pollock surimi (Soeda *et al*., 1996). Jiang *et al*. (2000a) investigated the properties of surimi gels from threadfin bream and pollack surimi set at 30°C or 45°C with MTGase from *Streptoverticillium ladakanum*. The optimal amount of MTGase and setting conditions were 0.3 unit/g surimi either at 30°C for 90 min or at 45°C for 20 min for threadfin bream. For pollack surimi, MTGase at a level of 0.2 unit/g surimi with setting at 30°C for 60 min were found to be optimal condition. MTGase catalyzed MHC cross-linking of both pollack and threadfin bream surimi and increased the gel forming ability of surimi. Ramirez *et al*. (2000) reported that optimal condition for the

addition of MTGase to improve gel quality from striped mullet (*Mugil cephalus*) involved: a concentration of MTGase of 9.3 g/kg of surimi, and setting at 37°C for 3.9 h. Under these conditions, the maximal shear strain was observed. However, the addition of MTGase at 5 g/kg of surimi in combination with setting at 34.5°C for 1 h rendered the maximal shear stress. Concentration of MTGase, temperature and time were also optimized to improve the mechanical properties of surimi from silver carp. Optimal properties were obtained by employing the following setting conditions: a concentration of MTGase of 8.8 g/kg of surimi, at 39.6°C for 1 h. Under these conditions, a surimi gel from silver carp with shear stress of 146 kPa and shear strain of 1.59 was obtained. Shear stress was strongly influenced by temperature and time, while shear strain was moderately affected (Ramirez *et al*., 2000).

The use of MTGase in combination with other additives or techniques can improve the gel strength of surimi to different degrees. Jiang *et al*. (1998a) investigated the effect of combining MTGase from *Streptoverticillium ladakanum* with ultraviolet (UV) irradiation on the gelation of minced mackerel. The gel strength of minced mackerel with MTGase alone at a concentration of 0.47 unit/g was greater than that of control. When MTGase-supplemented minced mackerel was exposed to UV light for the optimal irradiation time of 20 min, the gel strength could be further increased by 25%. MTGase causes the cross-linking of MHC of mackerel actomyosin and UV irradiation enhances this polymerization of MHC by MTGase. Accordingly, the gel strength of minced mackerel increased significantly when MTGase and UV irradiation were used in combination.

A combination of MTGase, reducing agent and protease inhibitor was employed to improve the quality of underutilized fish surimi. MTGase could catalyze

the MHC cross-linking and increase the gel forming ability of hairtail surimi. The texture degradation caused by the endogenous proteases could be inhibited by the addition of inhibitor. The best solution to improve gel-forming ability of frozen hairtail surimi was the combination of 0.35 units MTGase/g, 0.1% sodium bisulfite and 0.01 mM E-64 (Jiang *et al*., 2000b).

8. Phosphate compounds

Phosphates are compounds prepared from phosphoric acid where the acid has been partially or fully neutralized with alkali metal ions, predominately sodium, potassium, or calcium (Dziezak, 1990). Phosphates can be divided into two general classes:

1. Orthophosphates

Orthophosphate is the larger and perhaps more important group to the food industry. It consists of one phosphorus atom tetrahedrally surrounded by four oxygens (Table 2). It can form straight-chain and cyclic polymers. These compounds have three valences that can be filled by hydrogen atoms, alkali metal cations, or a combination of hydrogens and metal cations. Monobasic orthophosphates have one alkali metal ion and two hydrogens; dibasic orthophosphates have two metal ions, one hydrogen; and tribasic orthophosphates are fully neutralized with three metal ions (Dziezak, 1990).

Table 2. Classes, formulas, pH, solubility, and functions of several phosphates.

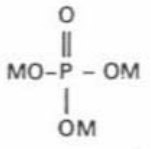
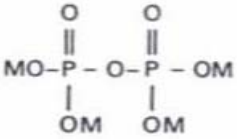
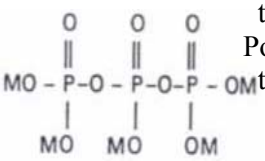
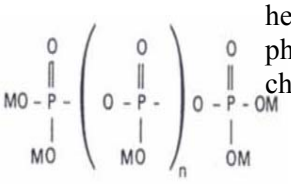
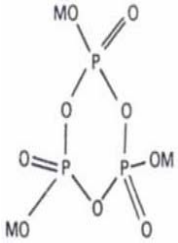
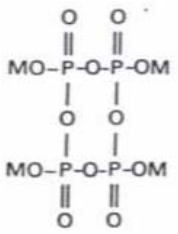
Class of Phosphate and Basic structure[a]	Phosphate name	Generally accepted formula	pH (1% solution	Solubility at 25°C (g/100g water)	Functions
Orthophosphates	Monosodium phosphate	NaH_2PO_4	4.6	87	Emulsifier, buffer
	Disodium phosphate	Na_2HPO_4	9.2	12	Emulsifier, buffer
	Disodium phosphate dehydrate	$Na_2HPO_4 \cdot 2H_2O$	9.1	15	Emulsifier, buffer
	Trisodium phosphate	Na_3PO_4	11.8	14	Emulsifier, buffer
	Monopotassium phosphate	KH_2PO_4	4.6	25	Water binding in meats
	Dipotassium phosphate	K_2HPO_4	9.3	168	Emulsifier, buffer
	Tripotassium phosphate	K_3PO_4	11.9	107	Emulsifier, buffer
	Monocalcium phosphate	$Ca(H_2PO_4)_2$	3.8	-	Acidulant, leavening acid, dough conditioner, yeast food, nutrient
Condensed phosphate					
Pyrophosphates	Sodium acid pyrophosphate	$Na_2H_2P_2O_7$	4.3	15	Emulsifier, buffer, sequestrant, water binding agent in meats
	Tetrasodium pyrophosphate	$Na_4P_2O_7$	10.3	8	Dispersant, coagulant, crystallization inhibitor in canned tuna
	Tetrapotassium pyrophosphate	$K_4P_2O_7$	10.5	187	Emulsifier, water, binding agent in meats, suspending agent
Tripolyphosphate	Sodium tripolyphosphate	$Na_5P_3O_{10}$	9.9	15	Emulsifier, water binding agent in meats
	Potassium tripolyphosphate	$K_5P_3O_{10}$	9.6	193	Emulsifier, water binding agent in meats
Long-chain Polyphosphates	Sodium polyphosphates, glassy, or Graham's Salt; three chain lengths;	$(NaP0_3)_6 \cdot Na_2O$	7.7	40[b]	Sequestrant, emulsifier, water binding agent in meats, suspending agent
	sodium hexametaphos-phate has an average chain length of 13	$(NaP0_3)_{13} \cdot Na_2O$	6.9	40[b]	Sequestrant, emulsifier, water binding agent in meats, suspending agent
		$(NaP0)_{21} \cdot Na_2O$	6.3	40[b]	Sequestrant, emulsifier, water binding agent in meats, suspending agent

Table 2. (Continued)

Class of Phosphate and Basic structure[a]	Phosphate name	Generally accepted formula	pH (1% solution	Solubility at 25°C (g/100g water)	Functions
Metaphosphates Tri- 	Sodium trimetaphosphate	$(NaP0_3)_3$	6.7	23	
Tetra- 	Sodium tetrametaphosphate	$(NaP0_3)_4 \cdot 4H_2O$	6.2	18	

[a]M stards for one equivalent of a metal ion or hydrogen
[b]Solubility is higher than 40% but this value is recommended for ease of preparation and use
Source: Dziezak (1990)

2. Condensed phosphates

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 straight-chain phosphates called polyphosphates and rings, termed metaphosphates (Dziezak, 1990).

Of the polyphosphates, pyrophosphates are the simplest as they have a two-phosphorus chain. Tripolyphosphates are next in the series with three phosphorus atoms 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 polyphosphates

of varying chain lengths (Ellinger, 1977). Selection is based on their average chain length. Polyphosphates 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).

Chemical properties and functionality of phosphates

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

- Inactivation of metal ions and processing water treatment

The function of phosphate is a metal chelator (Park, 2000). The phosphates can inactivate metallic ions, which are capable of interfering with necessary food-processing reactions. They inactivate the metallic ions either by precipitating and removing them from interference with the desired food-processing reactions or by complexing and maintaining them in a soluble bound state (Ellinger, 1975).

The polyphosphates, including those ranging from two to many phosphate units per molecule, form soluble complexes with nearly all of the metallic ions. Thus, they are considered to undergo ion-exchange reactions, in which a hydrogen, sodium, or potassium ion is exchanged for one of the alkaline-earth or transition-metal ions (Ellinger, 1975). Very weak, soluble complexes are formed with alkali-metal and ammonium ions. More stable but somewhat dissociated complexes are formed with the alkaline-earth metals, such as calcium, magnesium. Very stable,

soluble complexes 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, and their absorption and retention may actually be increased 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.

- 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 charged nature, polyphosphates interact with various food constituents to produce many useful effects. 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 they increase water-binding and gel formation of the proteins, improve whipping properties (by increasing the solubility of the protein), and improve the precipitation and insolubilization of proteins for separation (Ellinger, 1972b; VanWazer, 1971). The polyelectrolyte properties of polyphosphates generally increase with chain length (Ellinger, 1972b).

- 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 acid pyrophosphate have the best buffering capacity (Van Wazer, 1971). Long-chain polyphosphates are generally poor buffers, and their buffering capacity decreases with increasing chain length. Phosphates 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 trisodium phosphates, sodium tripolyphosphate, and tetrasodium pyrophosphate are commonly used for this purpose (Dziezak, 1990).

Applications of phosphate compounds in muscle foods and surimi

Phosphates have been widely accepted as the potential additives in fish and seafood to improve the functional properties of those products by increasing water retention in fresh fish and reducing the thaw loss in frozen fish (Chang and Regenstein, 1997a). Phosphate is also added to surimi as a cryoprotectant with its function as a metal chelator and/or antioxidant. In addition, because of the strength of phosphate in raising pH, the water holding/binding of the gel can be improved and salt solubilization of myofibrillar proteins can be increased (Trout and Schmidt, 1983). The effectiveness of phosphates on water retention properties of meat products depends on the type of phosphates and the amount used (Shults *et al*., 1972; Trout and Schmidt, 1984, 1986; Lewis *et al*., 1986). Trout and Schmidt (1986) showed that the effectiveness of phosphates on prevention of cook loss of meat products was in the following order: pyrophosphate > tripolyphosphate > tetrapolyphosphate >

hexametaphosphate. The effects of phosphates on increasing water retention of muscle were summarized by Hamm (1971), involving the increases in pH and ionic strength, the binding of phosphates to meat proteins, and the dissociation of actomyosin into actin and myosin. Phosphate is normally added to surimi in combination with cryoprotectants such as sugar or sorbitol (Sultanbawa and Li-Chan, 2001). The raising pH caused by this compound results in the improved water holding/binding of the gel as well as better solubilization of myofibrillar proteins (Park, 2000). Pyrophosphate has been reported to dissociate protein complex, leading to the improved gel forming ability (Matsukawa *et al*., 1995). Although phosphate compounds have been proven as the promising processing aid, it probably shows the detrimental effect on gel property since it could chelate the Ca^{2+}-ion. This may impede the setting of surimi induced by endogenous TGase. Trout and Schmidt (1987) concluded that at high ionic strengths (>0.25), pyrophosphate affected hydrophobic interactions which stabilize the protein structure, and thus, the thermal stability of the protein. Yagi *et al*. (1985) confirmed that inorganic polyphosphate offered a high degree of protection (to carp myofibrils) from thermal denaturation. Water retention is correlated with increased pH and normally associated with the use of alkaline polyphosphates such as sodium tripolyphosphate. Orthophosphates have virtually no effect on water-binding (Offer and Trinick, 1983). Nielsen and Pigott (1994) reported that the gel strength of commercial surimi was increased by addition of phosphate blends. Further increases were obtained by complete replacement of sodium tripolyphosphate with blended phosphates. Phosphate blends also permit nonisothermal flow of surimi, allowing the application of extrusion machinery and processing techniques to produce final products at the site of surimi production (Nielsen and Pigott, 1994).

Objectives

1. To study the effect of type and concentration of phosphate compounds on setting and gel forming ability of surimi
2. To study the influence of phosphate compounds in combination with $CaCl_2$ or MTGase on setting and gel forming ability of surimi
3. To study the effect of phosphate compounds on some properties of muscle proteins