Chapter 1

Introduction

Freshness of fish is generally considered as the most crucial requirement for the raw material to be processed into surimi. Time and temperature of the fish between capture and processing can affect the final surimi quality (Park and Morrissey, 2000). Lower gel quality can be found with gel made from fish stored over time in ice. However, the rate of loss of gel strength appears to vary among species. The rate of decline in gel strength is dependent on the denaturation and extent of proteolysis of myofibrillar proteins. The gel strength of kamaboko made from lizardfish kept in ice for 3 days was 50 % of that made from fresh fish (Kurokawa, 1979). Northern squawfish surimi could be made from fish stored for up to 9 days (Lin and Morrissey, 1995). Surimi gel quality can be influenced by many factors affecting protein structure. Prolonged holding times and elevated temperatures can cause severe proteolysis of myofibrillar proteins, which is directly associated with inferior gel quality (Suzuki, 1981). Degradation of myosin heavy chain (MHC) generally occurred during iced storage of Pacific whiting (Benjakul *et al.*, 1997). During handling, leakage of digestive enzymes into the muscle also results in subsequent hydrolysis of muscle proteins. Therefore pretreatment of fish, including beheading and evisceration prior to handling, can be another means to retard the deterioration caused by proteolysis (Benjakul *et al.*, 2002).

Thailand is one of the largest surimi producers in Southeast-Asia. At present, 12 surimi factories are located in Thailand, with a total production of about 60,000 metric tons per year (Morrissey and Tan, 2000). Normally, threadfin bream (*Nemipterus spp.*), bigeye snapper (*Priacanthus spp.*), croaker (*Pennahia and Johnius spp.*) and barracuda (*Sphyraena spp.*) are species commonly used for surimi production in Thailand. Fresh or ice-stored fish are

commonly used for surimi production worldwide. Currently, surimi has prepared form fresh fish, either at sea on factory ships or on land. Land-based processing has restricted to the time when fresh fish are available and must be sited near the fishing grounds. Due to over exploitation and the lack of raw material, fish fleet has to go farther, leading to the poorer quality of raw material. This is mainly caused by degradation mediated by endogenous proteinase or microorganisms. Due to the insufficient amount of fresh fish, surimi industry has to use the low quality fish, which exhibit the inferior gel quality. The improvement of surimi gel quality either by development of new processing or by direct enhancing the gel strength has been paid more attention. The gel quality has been improved by various approaches such as appropriate setting as well as the addition of protein additives including oxidizing agents. Therefore, washing fish mince with media containing oxidizing agents at proper concentration would be a means to improve the surimi gel property. Addition of some additives in combination with oxidizing agent washing would achieve the propose of gel strengthening of surimi, especially from low quality fish.

Literature Review

1. Chemical composition of fish

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).

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

1.1 Myofibrillar protein

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

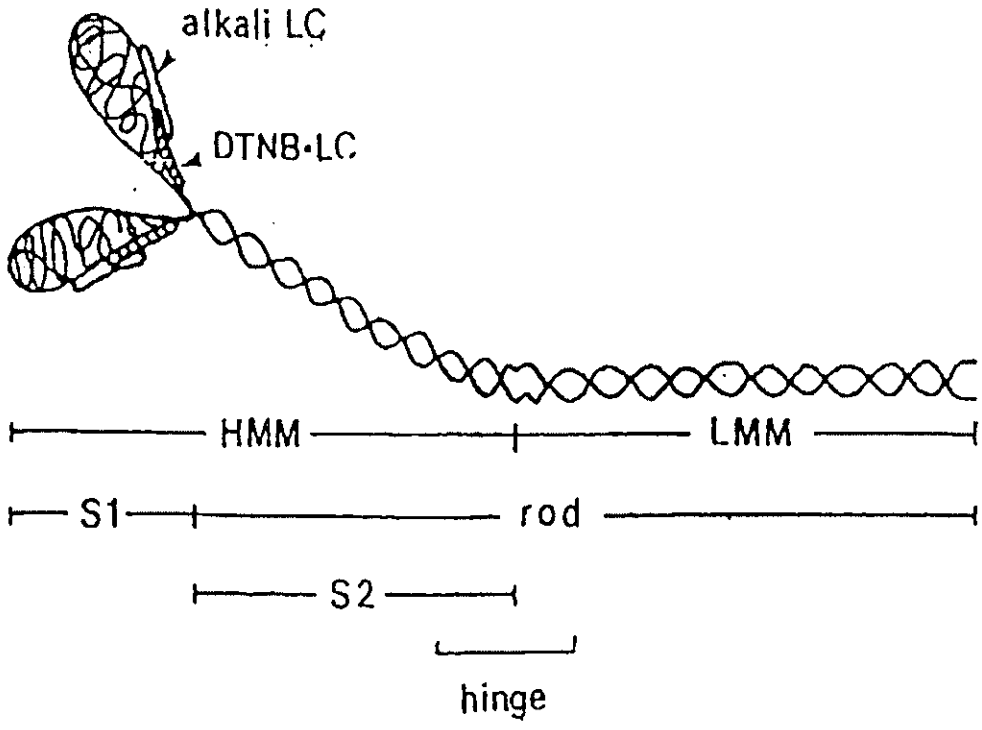

Figure 1 Model of myosin molecule

Source: Xiong (1997)

- Actin

Actin constitutes about 22 % of myofibrillar mass with a molecular weight of 42,000 daltons. Normally, actin in muscle tissue is associated with troponin and tropomyosin complex. It also contains a myosin binding site, which allows myosin to form temporary complex with it during muscle contraction or the permanent myosin-actin complex during rigor mortis in postmorterm (Xiong, 1997). Monomer form of actin is called G-actin and after polymerization, actin filaments are formed and referred to as F-actin. Two F-actin in helix form is called super helix (Foegeding *et al.*, 1996) (Figure 2).

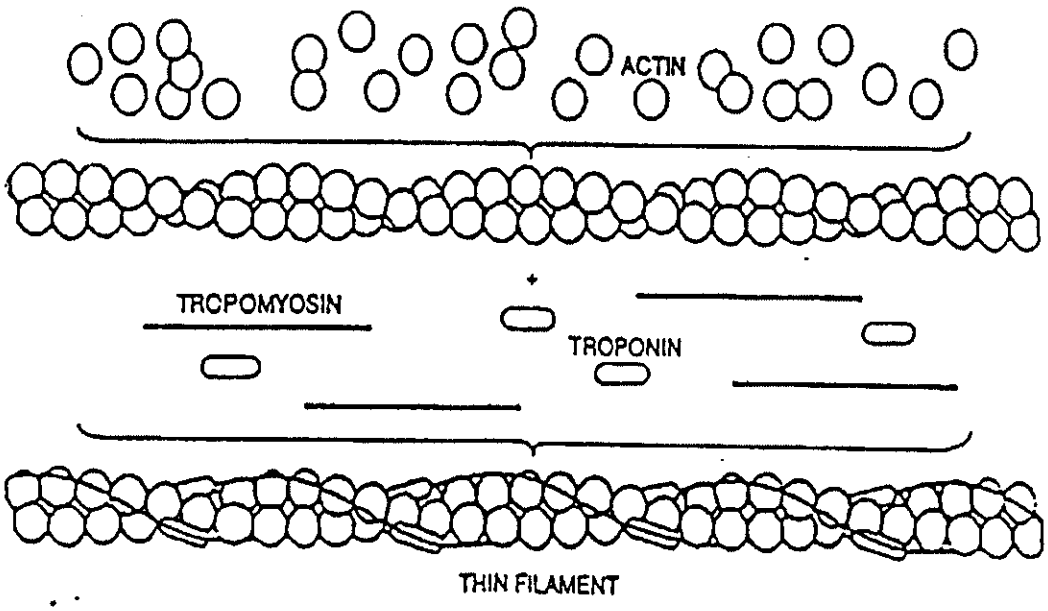

Figure 2 Structure of actin, troponin and tropomyosin
Source: Foegeding *et al.* (1996)

- Troponin

Troponin and tropomyosin regulate muscle contraction. Troponin accounting for 8-10 % of myofibrillar proteins consists of three subunits such as troponin C; which is a calcium binding protein and confers calcium regulation to the contraction process via the thin filament, troponin I; which strongly inhibits ATPase activity of actomyosin, and troponin T; which provides a strong association site for binding of tropomyosin (Foegeding *et al.*, 1996).

- Tropomyosin

Tropomyosin represents approximately 8-10 % of myofibrillar protein. It has two subunit chains (Suzuki, 1981). In skeletal muscle, two polypeptides, alpha- and beta-tropomyosin can combine to form a tropomyosin dimer. 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 monomers (Foegeding *et al.*, 1996).

1.2 Sarcoplasmic protein

Sarcoplasmic protein contain many kinds of water soluble proteins called myogen. It represents 20-35 % of the total content in muscle (Mackie, 1994). It can be obtained by simply pressing fish meat, or by extracting with low ionic strength salt solution (Mackie, 1994). The content of sarcoplasmic protein in fish meat varies with fish species, but is generally higher in pelagic fish such as sardine and mackerel and lower in demersal fish (Suzuki, 1981).

1.3 Stroma protein

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

2. Functional properties of fish protein

Fish myofibrillar protein has excellent functional characteristics such as emulsifying properties, gel-forming ability, and water holding capacity (Tanabe and Saeki, 2001; Lin and Park, 1996). Generally, fish myofibrillar protein is thermally and chemically less stable than that of other vertebrates (Yamashita *et al.*, 1978; Hashimoto *et al.*, 1982) and its functional properties are generally lowered when protein denaturation occurs (Regenstein *et al.*, 1983). The functional property of muscle protein varies with the product, processing method, and stage of processing (Xiong, 1997; Smith, 1988). Additionally, the functional properties of proteins are governed by primary, secondary, tertiary and quaternary structural components of the molecules (Pomeranz, 1991).

2.1 Water holding capacity

Myofibrils are composed of 25 % protein and 75 % water. Therefore, the majority of water in meat is confined within the myofibrils in the spaces between the myosin and actin (Xiong, 1997). Myofibrils are the primary sites for intracellular water. Other cellular components may also contribute to water-binding in the meat. Entrapment and mobility of the water present outside the cell could be affected by many environmental factors, and water content varies with different processing treatments (Xiong *et al.*, 2000). There are two major types of forces that contribute to water retention in meat: polarity, including surface charges, and capillary effects. Binding of water to the surface of protein through hydrogen bonds between water molecules and charges and dipolar amino acid residues seems to be insignificant for water retention in meat. Any change in the surroundings of myofibrils that results in increased protein charges or dipoles (high concentrations of salt and pH away from the protein isoelectric point) would lead to increase water retention in meat (Xiong, 1997).

2.2 Solubility

Solubility of proteins is of a primary importance for the manufacture of processed muscle foods, including comminuted, restructured, and formed meats (Xiong, 1997). Most functional properties of muscle proteins are related to protein solubility (Lin and Park, 1996). The solubility of a protein under a given set of environmental conditions is the thermodynamic manifestation of the equilibrium between protein-protein and protein-solvent interactions (Lawrence *et al.*, 1986). It is related to the net free energy change arising from the interactions of hydrophobic and hydrophilic residues of the protein with the surrounding aqueous solvent. The lower the average hydrophobicity, the higher the solubility is obtained (Damodaran, 1996). Decrease in solubility during frozen storage and loss of ATPase activity was described for myosin in frozen fish (Li-Chen *et al.*, 1985). Since the solubility in high ionic strength media of the protein is impaired with the progress of protein denaturation, the solubility

of muscle proteins in salt solutions has been used to assess the quality of fish meat (Regenstein *et al.*, 1983; Akahane *et al.*, 1984). The textural properties depend largely on protein solubility, especially in the case of fish (Colmenero and Borderiad, 1983).

2.3 Gel-forming ability

Gelation of myofibrillar proteins has been shown to be largely responsible for the textural properties of processed fish products (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 maybe visualized as three-dimensional matrixes or network in which water is entrapped (Pomeranz, 1991).

3. Protein oxidation

Muscle proteins not only are responsible for textural properties of meat products but also serve as an important source of essential amino acids. Muscle proteins, like other muscle components such as lipids and pigments, are vulnerable to oxidative attack during the processing and storage of muscle foods (Martinaud *et al.*, 1997; Wang *et al.*, 1997). Oxidative modifications often lead to alterations in muscle protein functionalities, including gelation, emulsification, viscosity, solubility, and water holding capacity (Smith, 1987; Decker *et al.*, 1993; Wan *et al.*, 1993; Xiong *et al.*, 1993; Srinivasan and Hultin, 1997; Wang and Xiong, 1998).

The susceptibility of muscle foods to oxidative processes stems from their relatively high concentrations of unsaturated lipids, heme pigments, metal

catalysts, and various other oxidizing agents (Jonhs *et al.*, 1989). Oxidation is also facilitated by components in the muscle (Xiong and Decker, 1995). Oxidizing agents present in muscle tissues are capable of generation a number of reactive oxygen species (ROS), which include free radicals, such as the hydroxyl radical (•OH), peroxyl radicals (ROO•), alkoxyl radical (RO•), superoxide anion radical (O_2•-), and thiyl radical (RS•), as well as non radical oxygen derivatives, such as H_2O_2, singlet oxygen, 4-hydroxy-2nonenal, and ketoamines (Stadtman and Berlett, 1997). Polypeptide backbone and many amino acid residue side chains are vulnerable to oxidative attack. The common changes in oxidized proteins are destructions of amino acids, unfolding, fragmentation, and cross-linkage of proteins, and formation of protein carbonyls. The oxidative modifications usually result in increased susceptibility of the proteins to digestive enzymes, which is important for preventing toxin buildup in living cells (Levine, 1989; Stadtman, 1990; Agarwal and Sohal, 1994).

Consistent with the observations in living tissues, similar oxidative changes have been noted in proteins from postmortem muscle tissues. Li and King (1996) reported considerable increases in the surface hydrophobicity of myosin oxidized by an Fe(II)/ascorbate system. Using gel electrophoresis, several studies showed degradation and polymerization of myofibrillar proteins incubated with different model oxidation systems closely resembling meat or processed meat conditions (Decker *et al.*, 1993; Martinaud *et al.*, 1997; Srinivasan and Hultin, 1997). Kamin-Belsky *et al.* (1996) demonstrated a decreased digestibility of H_2O_2/hemin-cross-linked myosin. Oxidized myoglobin could form long-lived radicals that were capable of oxidizing of many other proteins naturally present in the muscle cell (Irwin *et al.*, 1999).

Liu and Xiong (2000) studied physicochemical changes and in vitro digestility of chicken breast myosin oxidized with a nonenzymic free-radical-generating system ($FeCl_3$/H_2O_2/ascorbate). Oxidation caused fragmentation and

polymerization of myosin. Myosin polymers were cross-linked mainly through disulfide bonds. Hydroxyl radicals destabilized myosin, lowering its denaturation temperature by up to 4°C. Oxidized myosin also produced a new thermal transition in the 60-80°C temperature range, which could be attributed to the formation of disulfide-stabilized polymers. The proteolytic susceptibility of myosin to pepsin, trypsin, and chymotrypsin was increased by oxidation. After incubation with the oxidants for 24 h, the MHC band essentially disappeared. On the other hand, oxidation increased the formation of numerous high-molecular-weight protein bands, which appeared as smears of dark stains at the top of the separating gel. Apparently, oxidation caused disulfide cross-linkage of myosin to form polymers (Liu and Xiong, 2000). Myosin molecule has ~42 sulfhydryl groups, many of which can be readily accessed by chemical reagents (Hofmann and Hamm, 1978). Upon exposure to hydroxyl radicals, the sulfhydryl groups of MHC would be oxidized to form intermolecular disulfide bond. Thus, oxidation not only causes fragmentation of MHC but also cross-linking of MHC, producing oligomers or polymers. Bhoite-Solomon *et al.* (1992) reported that H_2O_2 alone could also cause myosin to form disulfide-cross-linked aggregates but did not induce fragmentation of myosin.

Though the intense oxidation, especially with the strong oxidizing agent, causes the detrimental effect on functionality of protein, mild oxidative modification of myosin fibrillar proteins could facilitate protein gelation (Srinivasan and Xiong, 1996). The oxidative effect on muscle protein is extremely complex. Oxidative factors either inherent to muscle or introduced through meat processing determine the level of oxidizing, which affects the functionality of protein.

4. Surimi and surimi production

Surimi is Japanese term for mechanically deboned fish flesh washed with water and mixed with cryoprotectants. 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). It is used as an intermediate product for a variety of fribicated seafoods, such as the crabs legs and flakes. Washing is the important step for surimi production. Washing not only removes fat and undesirable matters, such as blood, pigments and odorous substances (Suzuki, 1981), but also increases the concentration of myofibrillar protein. Myosin has been known as the dominant protein involved in gelation. The use of fresh fish for the surimi production is also essential since tissue autolysis by endogenous proteolytic enzyme during storage reduces the level of extractable actomyosin available for gelation (Benjakul *et al.*, 2002; Benjakul *et al.*, 2003).

Surimi can be produced from different fish species. The different processes are used with the particular purposes as follows (Park and Morrissey, 2000):

-Heading, gutting and deboning

Head and gut are removed and the belly walls are thoroughly cleaned before deboning the carcass. Fish can be filleted prior to deboning.

-Mincing

Deskinned and deboned fillets give cleaner minced meat because blood, membrane, and others contaminants have been removed. A headed and gutted carcass, on the other hand, results in a higher final yield of minced flesh, but the quality is relatively low.

-Washing and dewatering

The most important step of surimi processing to ensure maximum gelling, as well as colorless and odorless surimi, is efficient washing. Approximately two-third of minced fish meat is myofibrillar proteins, which is the primary component in the formation of three-dimensional gel structure. The

remaining one-third consists of blood, myoglobin, fat, and sarcoplasmic protein, which impede the final quality of the surimi gels (Lin and Park, 1997). Consequently, washing which removes the undesirable one-third, increases the quality of surimi by concentrating the functional myofibrillar protein and extends the frozen shelf life. The washing processing involves mixing minced with cold water (5°C) and removing water by centrifuging. This process is repeated two or three times. The number of washing cycles and the volume of water varies with fish species, freshness of fish, type of washing unit, and the desired quality of the surimi. During a batch process, the water/mince ratio varies from 5:1 to 10:1. After washing, water is removed to a level of 82-85 % moisture, which is similar to that found in fish fillet (Park and Morrissey, 2000).

The important quality factors associated with water are temperature, hardness or mineral content, pH, and salinity. The level of chlorination in the water should also be considered because of its bleaching and deodorizing effect (Lee, 1986). The water must be refrigerated to a temperature below which the fish muscle proteins can retain their maximum functional properties. Temperature of water can vary based on the thermostability of fish proteins. Warm water fish can, therefore, tolerate a higher water temperature than cold water fish without reducing protein functionality (Arai *et al.*, 1973). However, considering the changes in air temperatures during processing, the recommended water temperature for obtaining maximum quality is 5°C or less. Theoretically soft water with minimum levels of minerals, such as Ca^{2+}, Mg^{2+}, Fe^{2+} and Mn^{2+}, is recommended for washing. Hard water causes deterioration of texture and color quality during frozen storage. In addition, Ca^{2+} and Mg^{2+}, are responsible for the color change (Lee, 1990). Furthermore, the pH of the water must be maintained at approximately that of prerigor fish muscle tissue (6.8-7.0) to obtain higher water retention of gels (Park and Morrissey, 2000).

-Adding the cryoprotectants

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. A mixture (1:1) of sodium tripolyphosphate and tetrasodium pyrophosphate at 0.2-0.3 % is commonly used. Surimi blocks are placed in a contact plate freezer and held for approximately 2.5 h or until the core temperature reaches -25°C (Park and Morrissey, 2000).

5. Biological (Intrinsic) factors affecting surimi quality

5.1 Effects of species

Functional properties of surimi depend on composition but cannot generally be predicted from compositional analysis. In addition to Alaska pollock, a number of species are used as raw materials for commercial surimi processing. Depending on the species used, the functional and compositional properties of the surimi vary. An *et al.* (1994) identified the enzymes in Pacific whiting as cathepsins B, H and L, which behave differently with different environmental conditions, such as pH, temperature and ionic strength. Cathepsin B and H are easily washed off during surimi processing, whereas cathepsin L remains in the muscle tissue (An *et al.*, 1994; Park and Morrissey, 2000). Cathepsin L has an optimum temperature of 55°C and causes textural deterioration when the Pacific whiting surimi paste is slowly heated (An *et al.*, 1994). Muscle protein from two species of bigeye snapper showed the different gelling property. *Priacanthus tayenus* protein exhibited the larger aggregation stabilized by hydrophobic interaction and disulfide bond than *P. macracanthus* (Benjakul *et al.*, 2002).

5.2 Effects of freshness and handling

Freshness of fish is primarily time/temperature-dependent. Generally, surimi produced from fish storage in ice for a larger time showed the decrease in gel-forming ability. Bigeye snapper and lizardfish kept in ice with extending storage time had the continuous decrease in breaking force and deformation (Benjakul *et al.*, 2002; Benjakul *et al.*, 2003). On at-sea vessels, processing of Alaska pollock occurs within 12 h, whereas at shore-side operations processing occurs within 24-48 h. Because of endogenous enzymes activated by rising temperatures, Pacific whiting is processed within a shorter period; immediately on at-sea vessels and within 20 h after harvest at short-side plants (Park and Morrissey, 2000).

The biochemical and biophysical changes during the development of rigor mortis induce significant changes in the functional properties of muscle proteins. Fish should be processed as soon as possible after going through rigor. Before passing through this stage, about 5 h in the case of pollock, it is difficult to remove the odor, various membranes, and other contaminants that affect product quality (Pigott, 1986). The length of time that fish can be held in ice or refrigeration before processing varies, depending on the species. Time and temperature of the fish between capture and processing can be considered two of the most important factors in final surimi quality. Holding temperatures at 4-6°C can make a significant difference in surimi quality compared with fish held close to 0°C (Park and Morrissey, 2000). Degradation of MHC was also affected by storage temperatures. Fish kept at 5°C showed higher degradation than those stored at 0°C, suggesting that ice water was more efficient than refrigeration in controlling proteolysis. When temperatures increased further, degradation occurred more rapidly (Park and Morrissey, 2000).

Proteolysis can take place at low temperature. With prolonged storage time, severe degradation occurred, although the storage temperature had been maintained at 0°C (Lin and Park, 1996). According to An *et al.* (1994), in the

temperature range of 0-5°C, the activity of cathepsin L was insignificant, whereas cathepsin B exhibited half of its maximal activity and cathepsin H retained about a fifth of its maximal activity. Therefore, cathepsins B and H might contribute to the degradation occurring at low-temperature storage. Consequently, to minimize proteolysis, fish should be processed promptly on landing or kept at 0°C if holding is necessary.

6. Chemistry of surimi gelation

Gelation is an orderly aggregation of proteins, which may not be denatured, forming a three-dimensional network (Hermansson, 1979). Samejima *et al.* (1981) proposed that the heat-induced gelation of myosin consist of two reactions: (1) aggregation of the globular head segments of the myosin molecules, which is closely associated with the oxidation of sulfhydryl groups and (2) network formation resulting from the unfolding of the helical tail segment. In addition, the head portions associates to form "super-junctions" which provide extra cross-linking to the gel network. Agents that block formation of disulfide bond did not prevent the aggregation of the myosin head but only retarded its onset. Another aggregation was possibly due to intermolecular association of side chains, which superimposes on the sulfhydryl-dependent reaction (Samejima *et al.*, 1981).

During heating of surimi pastes, the proteins unfold, exposing reactive surfaces of neighboring protein molecules, which then interact to form intermolecular bonds (Lanier, 2000). Chan *et al.* (1992) reported that thermal aggregation of fish myosin was coincidental with an increase in the surface hydrophobicity of the unfold domains of myosin molecules and was affected by the temperature at which these domains unraveled. Extent of aggregation for fish myosin seems to depend on the amount of hydrophobic surface exposed on the headed molecules (Chan *et al.*, 1992; Wicker *et al.*, 1986). 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. Covalent bonds such as disulfide bonds are dominant when heating at high temperatures (>40°C) (Lanier, 2000). However, a variety of crosslinks, including ε-(γ-glutamyl)lysine [ε-(γ-Glu)Lys] crosslinks and crosslinks through aldol condensation, are believed to be closely related to texture (Sakamoto *et al.*, 1995). The formation of ε-(γ-Glu)Lys crosslinks by the catalytic action of transglutaminase (TGase) have been reported (Ikura *et al.*, 1980; Motoki and Nio, 1983; Kurth and Rogers, 1984; Kato *et al.*, 1991).

There are mainly four types of bonds which contribute to the building of a network structure during the gelation of a surimi paste: salt linkages, hydrogen bond, disulfide bond, and hydrophobic interactions (Niwa, 1992).

6.1 Salt linkages and hydrogen bonds

The intermolecular salt linkages which occur between charged amino acids of the protein chain are thought to play an important role in the stabilization of the network structure of various food gels. Additionally, there are many amino acids capable of forming intermolecular hydrogen bonds. The tyrosin, serine, hydroxyproline, and threonine residues contain a hydroxyl group, and the proline and hydroxyproline residues contain an imino group, both of which act as proton donors and acceptors, while glutamine and asparagine residues both contain a large number of imino and carbonyl groups in the polypeptide chain (Niwa, 1992). Hydrogen bonds are important in the stabilization of bound water within the hydrogen. A large amount of water molecules are hydrogen bonded to the polar amino acid residues, which are abundantly exposed on the molecular surface of the proteins (Niwa, 1992).

6.2 Disulfide bonds

An intermolecular disulfide (S-S) bond is formed by the oxidation of two cysteine residues:

$$\text{Protein-SH} + \text{HS-Protein} \xrightarrow{O_2} \text{Protein-S-S-Protein}$$

Earlier it had been thought that the formation of S-S bonds upon heating of surimi pastes did not occur in the absence of oxidant because abundant cysteine residues remain on the molecular surface of myofibrillar proteins in the cooked gel (Niwa and Nakayama, 1961) and the oxidation-reduction potential of cooked surimi gels is relatively low (Yokozeki, 1959). The S-S bonds are formed in the inner part of the protein, since urea and guanidine hydrochloride are known to dissipate the hydrophobic interactions stabilizing the inner structure of protein (Itoh *et al.*, 1980). Furthermore, it is assumed that disulfide interchange occurs during heating of the surimi paste. Intramolecular S-S bonds are converted to intermolecular S-S bonds. This is because surimi gels are strengthened not only by addition of oxidant prior to heating, but also by such addition of reducing agents as ascorbic acid and cyteine (Itoh *et al.*, 1980).

The aggregation of the head portions of the myosin molecules, in which the sulfhydryl groups are located, is mainly through disulfide bond formation. This is followed by cross-linking of the rod portion myosin molecules which accompanies the conversion of the ∝-helix to a random coil. Such a contribution of the sulfhydryl groups to gelation was suggested from the finding that the thermally induced increase in rigidity and turbidity of myosin subfragment S1 and heavy meromyosin (HMM) solution was suppressed upon the addition of dithiothreitol, and that SH content was remarkably decreased upon heating the S1 fragments (Samejima *et al.*, 1981). It has been demonstrated that the formation of S-S bonds is more intensive for carp (Itoh and Ikeda, 1979) and Atlantic croaker actomyosin (Liu *et al.*, 1983) at the higher temperatures of cooking (80°C or above) than at the lower temperatures at which setting occurs. Benjakul *et al.* (2001) also reported that disulfide

bonds were found in bigeye snapper actomyosin during thermal gelation process.

6.3 Hydrophobic interactions

About 25 % of amino acids that constitute the myosin molecule are hydrophobic amino acids such as alanine, valine, leucine, isoleucine, proline, tryptophan, and phenylalanine (Tsuchiya and Matsumoto, 1975). If these hydrophobic residues are in contact with water molecules, a so-called "clathrate" or "iceberg" is formed, whereby a large number of water molecules are hydrogen-bonded to one another around the residue (hydrophobic hydration). However, such an ordering of water molecules in this way is not stable thermodynamically. If the temperature rises, hydrogen bonds become less stable and hydrophobic hydration becomes favored. Thus the hydrophobic amino acid residues become more exposed and subsequent hydrophobic interaction occurs.

During the slow-setting upon incubation near 40°C, hydrophobic groups are introduced onto their molecular surface and hydrophobic interactions proceed and play an important role in the setting phenomenon (Niwa *et al.*, 1981). Benjakul *et al.* (2001) reported that the increase in surface hydrophobicity of actomyosin from bigeye snapper during thermal gelation suggesting that hydrophobic interaction involved in gelation.

6.4 Other bonding mechanisms

Covalent cross-linking reactions other than disulfide bonding has also been shown to occur in surimi gel and occur at a rate corresponding to the rate of gelation as measured rheologically (Kamath *et al.*, 1990). Appearance of higher molecular weight polymers of Alaska pollack MHC corresponded to its disappearance of MHC during setting. At 25 °C, the optimum setting temperature for pollock, the cross-link formation of MHC is the most pronounced. However, cross-link formation is also evident in sols incubated at 4°C and 40°C (Niwa, 1992). Gelation of the surimi sol also occurs during

incubation at 50°C; however, such gelation evidently does not involve the non-disulfide cross-linking of myosin (Niwa, 1992).

The mechanism of the cross-linking reaction has recently been proven to be attributed to the action of transglutaminase (Seki *et al.*, 1990). Its activity may be species-specific, but is also likely related to the surface conformation of the substrate, as determined by the heat stability of the particular myosin (Niwa, 1992). It was reported that transglutaminase may require a hydrophobic region on one of the substrate molecules for cross-linking reaction (Niwa, 1992).

7. Setting of surimi gel

Setting or suwari is a well known occurrence in the surimi pasts during the incubation at temperatures lower that 40°C. This phenomenon involves network formation of myosin due to the cross-linking induced by endogenous transglutaminaese (TGase) (Seki *et al.*, 1990; Tsukamaza *et al.*, 1993). TGase has been reported to catalyze an acyl transfer reaction between γ-carboxamide groups of glutamyl residues in proteins as the acyl donors and a variety of primary ammine and water as the acyl acceptor (Kumazawa *et al.*, 1995). 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).

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 has been found to be 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). 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).

8. Softening of surimi gel

Gel softening or gel weakening termed "modori" in a problem found in surimi, especially for some species. This is associated with degradation of muscle proteins caused by the endogenous heat-activated proteinase (An *et al.*, 1996; Benjakul *et al.*, 1997; Jiang *et al.*, 2000). Proteolytic activity in muscle is high at temperature above 50°C and causes the rapid and severe degradation of myofibrillar proteins, particularly myosin (Wasson *et al.*, 1992). Such proteolytic degradation of myofibrillar proteins has a detrimental effect on surimi quality, and substantially lowers the gel strength (Morrissey *et al.*, 1993). Among the numerous proteinases present in muscle, cysteine endoproteinases have the most serious effect on texture because of their thermal stability and ability to cleave internal peptide bonds (Kirshke and Barrett, 1987). Proteinases associated with gel weakening can be categorized

into two major groups: cathepsin (Seymour *et al.*, 1994; Toyohara *et al.*, 1993) and heat-stable alkaline proteinase (Wasson *et al.*, 1992).

High level of cysteine proteinase activity mediated by cathepsin B, H, and L have been found in Pacific whiting and arrowtooth flounder (An *et al.*, 1994; Wasson *et al.*, 1992), chum salmon during spawning migration (Yamashita and Konagaya, 1990), and mackerel (Lee *et al.*, 1993). Softening of arrowtooth flounder is due to a cysteine proteinase that has maximum autolytic activity at 50-60°C (Greene and Babbitt, 1990). When Pacific whiting muscle was incubated at 60°C for 30 min before cooking at 90°C, most of MHC was degraded, the resultant surimi gel did not have measurable gel strength (Morrissey *et al.*, 1993).

9. Ingredient in surimi

Gelation of fish proteins is the most important step in forming desired texture in many seafood products, particularly those from surimi. To strengthen the gel, various chemical additives as well as enzyme have been successfully used in surimi.

9.1 Microbial Transglutaminase

During setting of surimi sol, enzymatically catalyzed formation of nondisulfide covalent bonds between protein molecules generally occurs. Those bonds form between the amino acids glutamine and lysine [ε-(γ-glutamyl)lysine dipeptide crosslinks], each on a neighboring protein chain, from the action of the endogenous transglutaminase (TGase)(protein-glutamine γ-glutamyltransferase; EC 2.3.2.13) (Nowsad *et al.*, 1994). TGase can catalyze the acyl transfer reaction between γ-carboxyamine groups of glutamine residues in proteins, peptides, and various primary amines. In the absence of primary amines, water may act as the acyl acceptor, resulting in deamination of γ-carboxyamine groups of glutamine to form glutamic acid (Ashie and Lanier, 2000) (Figure 3).

TGase is a sulfhydryl enzyme with a conserved pentapeptide (Tyr-Gly-Gln-Cys-Trp) active site sequence and is readily inactivated by sulfhydryl reagents (such as *N*-ethylmaleimide and *p*-chloromercuribenzoate), which alkylate free sulfhydryl groups (Folk, 1983). Cations such as Cu^{2+}, Zn^{2+}, and Pb^{2+} also inhibit TGase activity by binding to the thiol group of active site cysteine residue (Seguro *et al.*, 1995). TGase has been identified in tissue of various species including mammals, birds, fish and shellfish, plant and microorganisms. Recently, TGase from microorganisms or microbial TGase (MTGase) have been successfully produced by fermentation process. TGase from *Streptoverticillium mobarense* is Ca^{2+} in-dependent in a hydroxamate assay (Ajinomoto, 1998) and also differs from mammalian TGase in molecular weight, thermal stability, isoelectric point, and substrate specificity (Ando *et al.*, 1989; Tsai *et al.*, 1996). MTGase can be obtained from *Streptoverticillium ladakanum* and *Streptoverticillium mobaraense* (Seki *et al.*, 1998; Shann *et al.*, 1998).

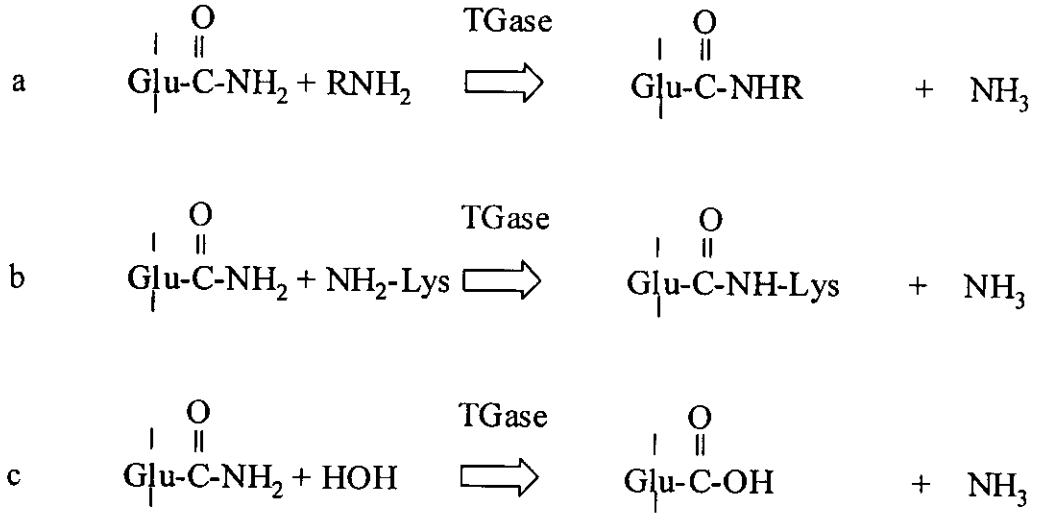

Figure 3 Reaction catalyzed by TGase

Source: Ashie and Lanier (2000)

MTGase offers a means of upgrading the gelling quality of surimi (Ando *et al.*, 1989). Lanier and Kang (2000) reported that, in Pacific whiting gels, the addition of MTGase has more pronounced effects, especially when combined with beef plasma (1 %), which inhibits heat stable protease. TGase from *Streptoverticillium ladakanum* and *Streptoverticillium mobaraense* catalyzed the crosslinking of fish myosin heavy chain and substantially increased the gel strength of mackerel surimi (Tsai *et al.*, 1996), threadfin bream surimi, pollock surimi (Jiang *et al.*, 2000), silver carp surimi (Ramirez *et al.*, 2000) and Alaska pollock (Seguro *et al.*, 1995). Addition of MTGase to surimi significantly increased the gel strength, particularly when the surimi has lower natural setting ability (Lee *et al.*, 1997; Seguro *et al.*, 1995; Kumazawa *et al.*, 1993). An increase in non-disulfide polymerization and formation of [ε-(γ-glutamyl)lysine isopeptides was found with an increase in setting time and MTGase concentration (Tsukamasa and Shimizu, 1990; Benjakul and Visessanguan, 2003). Jiang *et al.* (2000) found that the breaking force and deformation of the golden threadfin bream gels reached a maximum when 0.3 unit MTGase/g surimi was added. When the concentration of MTGase higher than 0.4 unit/g gel was used, the decreases in gel strength was found and the gels became rigid and brittle. The highest gel strength of pollock surimi was obtained with the addition of 1 to 2 units MTGase/g (Sakamoto *et al.*, 1995), however, the optimal MTGase for minced mackerel was 0.34 unit/g (Tsai *et al.*, 1996). According to Jiang *et al.* (1998), the gel strength of minced mackerel increased with the increase of MTGase up to 0.47 unit/g. Therefore, the amount in MTGase added highly depended on fish species and also other factors such as freshness, protein quality, and harvesting season (Asagami *et al.*, 1995).

9.2 *L*-ascorbic acid

L-ascorbic acid (AsA) is commonly used in bread dough to improve textural properties by the formation of –S-S- bonds through the oxidation of sulfhydryl (-SH) groups (Park, 2000). It is known that the addition of *L*-

ascorbic acid to surimi results in an increased gel strength in heat-induced surimi gel (Nishimura *et al.*, 1996). Thus, *L*-ascorbic acid is widely used as an improver in kamaboko manufacture. The improvement mechanism has been explained in the way that after being added to surimi, *L*-ascorbic acid is rapidly oxidized to dehydroascorbic acid (DHA) by oxidase or other factors, and DHA has been proposed to oxidize sulfhydryl groups on muscle proteins to disulfides and to form intermolecular bonds. (Yoshinaka *et al.*, 1972). Oxidized glutathione (GSSG), which is produced by DHA reductase in flour, has been suggested to catalyze disulfide bridge-formation among flour proteins. (Nicolas *et al.*, 1980). DHA acts as an oxidant to provide GSSG, but does not directly oxidize thiols in proteins to disulfides. It has not been clarified whether the same mechanism works in the gel formation of fish meat. Nishimura *et al.* (1996) found that *L*-ascorbic acid significantly enhanced the decrease in the amount of MHC from fish gel, by promoting the polymerization of MHC *via* disulfide bridging.

The hydroxyl radical, derived from H_2O_2, is a potent oxidant, but it did not contribute much to Cys oxidation, most probably because the concentration of the hydroxyl radical was too low. Once the thiyl radical ($RS^{\bullet}$) has been formed, the radical chain reaction propagates to form $RSOO^{\bullet}$, $RSSR^{-\bullet}$, O_2^{-}, and $RS^{-\bullet}$ radicals. This chain reaction is terminated by the following reactions, finally to form disulfides (RSS) (Saez *et al.*, 1982).

$$2RS^{\bullet} \longrightarrow RSSR$$

$$RS^{\bullet} + RSSR^{-\bullet} \longrightarrow RSSR + RS^{-}$$

Based on the high rate constants (10^8-10^9 $M^{-1}s^{-1}$) of these disulfide-forming reactions (Adams *et al.*, 1969), the polymerization of MHC by this mechanism is eminently possible, if the steady-state concentration of protein thiyl radicals is sustained by the continuous supply of O_2^{-}. Thus, the addition of

L-ascorbic acid to surimi enhanced the oxidation of protein sulfhydryl groups to form disulfide-bridged polymers by producing the superoxide radical.

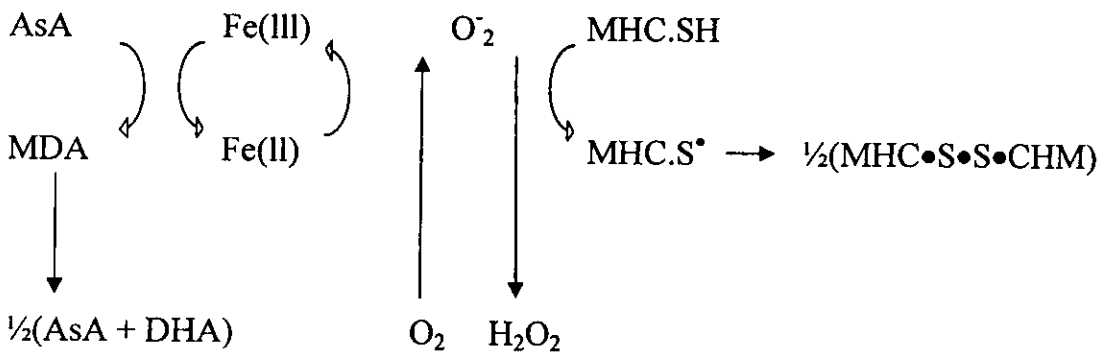

Figure 4 Scheme for the superoxide radical-dependent polymerization of MHC. Source: Nishimura *et al.* (1996)

9.3 Calcium compounds

Calcium compounds are commonly added as a gel enhancer. Yamamoto *et al.* (1991) used a mixture of sodium bicarbonate, calcium citrate, and calcium lactate as gel quality-improving agents. The levels of calcium lactate, calcium citrate, calcium sulfate, and calcium caseinate vary from company to company (i.e., 0.1-0.3 %) (Park, 2000). Low gel strength of Pacific whiting surimi compared with Alaska pollock surimi was thought to be due to a lower concentration of calcium ions in the flesh. Gordon and Roberts (1977) reported that the calcium content of Pacific whiting was 8.7 mg/100g meat, whereas pollock contained 63 mg calcium ions per 100 g meat (Sidwell, 1981). Lee and Park (1998) studied the effects of calcium compounds on the gel functionality of Pacific whiting and Alaska pollock surimi at three different thermal treatments. Calcium acetate, calcium chloride and calcium caseinate were very soluble, when the others were less soluble. In addition, calcium lactate showed

the highest solubility at a concentration of 0.2 %, whereas the solubility of the other compounds, in descending order. Shear strain values, however, were not affected by adding calcium compounds regardless of species and thermal treatments (Park, 2000). Lee and Park (1998) concluded that the textural properties of surimi can 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. Benjakul *et al.* (2004) also found that addition of $CaCl_2$ increased the breaking force and deformation of gel from bigeye snapper, threadfin bream, barracuda and bigeye croaker surimi. The gel strengthening effect was depending upon the concentration used.

10. Oxidizing agent

Many oxidizing agents have been used in food processing such as various sanitizing agent commonly used for cleaning. Hydrogen peroxide (H_2O_2) is clear, colorless, waterlike in appearance, and has a slightly pungent odor. At high concentrations, it has a slightly pungent or acidic odor. Hydrogen peroxide has been commercially available since the 1800's when it was used primarily for bleaching straw hats. Hydrogen peroxide's bactericidal and sporicidal properties have made it a useful tool in the food processing industry for equipment and packaging in aseptic systems. Hydrogen peroxide can also be considered bactericidal at low concentration. However, hydrogen peroxide is one of the most potent oxidizers and is more powerful than potassium permanganate, chlorine, or chlorine dioxide.

Sodium hypochlorite (NaOCl) has been one of the most widely used sanitizers. Hypochlorite (chlorine) has been used as a disinfectant for more than 100 years. Anti-microbial kinetics and its anti-microbial behavior of NaOCl to biofilm cell has been reported (Dodds *et al.*, 2000). Utilization of sodium hypochlorite in cleaning of chicken processing equipment in slaughterhouse

was investigated by Rossoni and Gaylarde (2000) to control the growth of *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus*. However, NaOCl is highly unstable, and the available chlorine in the solution rapidly decreases during storage. They are the most commonly used disinfectants for drinking water (Margolin, 1997).

Objectives

1. To study the effect of washing with various oxidizing agents on chemical composition, physicochemical properties of muscle proteins and gel-forming ability of surimi produced from low quality fish.
2. To study the effect of MTGase and some chemicals on gel improvement of surimi produced by oxidizing agent washing.
3. To investigate the effect of washing with oxidizing agent on setting phenomenon of surimi.