Surimi Enzymology and Biotechnology

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Abstract

The mechanisms of gelation, proteinase effects on gel softening and the nature of changes associated with surimi quality are complex. Based on the results thus far studied, this review will focus on the proteinases/inhibitors and transglutaminase (TGase and MTGase) effects on the quality of surimi. Seafood industry might be interested in how to prevent the deterioration of protein gels and consequently keep the quality of surimi-based products. Developments of some economic bio-productions of natural inhibitors and MTGase make the prevention of gel-softening on surimi-based process possible. Therefore, production of MTGase and cystatin, an inhibitor of cysteine proteinases, using biotechnology and their effects on inhibition of surimi gel softening will also be discussed.

Introduction

Surimi made from water-washed fish mince and mixed with cryoprotectants has a unique ability to form elastic gels through the interaction of myosin molecules (Lee et al., 1997). Gel-forming of myosin occurs at two stages during low temperature setting (0~45°C) and high temperature (90°C) heating (Hashimoto and Arai, 1978, Boye and Lanier, 1988). Low temperature setting is responsible for the polymerization of myosin heavy chain (MHC) association with the actions of TGase (Nowsad et al., 1993, 1994) and with the oxidation of SH groups (Jiang et al., 1986). However, gel disintegration frequently occurs during heating process (jiang et al., 1998).

Gelation (SUWARI) of surimi

Surimi gels are composed of a three dimensional protein network formed mainly by actomyosin. For elucidating the heat-induced gelation mechanism, actomyosin, myosin and myosin subfragments have been extensively studied for protein-protein interactions occurring in surimi gels (Wu et al., 1985a, b; Beas et al., 1988; Sano et al., 1993, 1995). According to these studies, myosin is the most important component in the formation of gel products. Sano et al. (1988) found that the gelation of carp actomyosin took place in two stages, i.e., at 30-41°C and 51-80°C as observed by dynamic viscoelastic behavior and differential scanning calorimetric (DSC) analysis. Differential shear modulus for natural actomyosin from Argentinean hake (Merluccius hubbsi) also produced two transitional temperatures ranges at 36-38°C and 48°C (Beas et al., 1988). Sano et al. (1990) thus proposed that the developments of gel elasticity at the 1st and 2nd stages are due to the interactions among tails and among the myosin heads, respec-
tively. Ziegler and Foegeding (1993) further summarized that gelation of myosin proceeds by losing its noncovalently stabilized $\alpha$-helical structure due to heating and then by increasing turbidity due to intermolecular association, which leads to the formation of a rigid protein network structure that is stabilized by covalent disulfide bonds and various noncovalent interactions.

Gelation characteristics of surimi such as firmness, cohesiveness, and water-holding capacity can be increased by incubating surimi paste below 40°C (also referred to as "setting" or "suwari") (Kimura et al., 1991). Suwari can be achieved within a short period of time (2-4 h) near 40°C (high-temperature setting) or within an extended period (12-24 h) below 40°C (low-temperature setting). Low-temperature setting is related to transglutaminase activity, while high-temperature setting is related to the transition in rheological properties of actomyosin observed at 36-38°C (Wu et al., 1985). $\alpha$-Helical structure of myosin, prevalent in the tail portion, unfolded at 30-40°C corresponding to low temperature setting (Ogawa et al., 1993). Lanier (1986) also observed a great increase in gel strength and, to a smaller extent, the elasticity of Atlantic croaker and sand trout surimi gels preincubated at 40°C. Furthermore, of the 14 fish species studied, the gel strength was highly correlated with the decrease in $\alpha$-helicity (r=0.85), therefore, Ogawa et al. (1995) proposed that the setting of surimi is initiated by the unfolding of $\alpha$-helix.

Gel strength of myofibrillar proteins can be influenced by factors affecting myosin structure. The gelation properties of myosin are highly related to the length of the double stranded $\alpha$-helical tail. Myosin rod (140 nm long) showed higher rigidity than light meromyosin (80 nm long) at all salt concentrations studied (Ishioroshi et al., 1982). Accordingly, proteolysis of myosin was shown to lower surimi gel strength (Morrissey et al., 1993). The native conformation of myosin is of primary importance for proper gelation. Maximum gel strength cannot be obtained if myosin is denatured or degraded prior to initiation of gelation (Niwa, 1992).

Softening (MODORI) of surimi-based products

Temperature plays an important role in surimi gelation. Fish muscles from various species revealed similar responses to temperature (Shimizu et al., 1981), i.e. a structure-setting reaction for gelation below 40°C (suwari) and a structure disintegration reaction at 50-70°C (modori). Low temperature setting is associated with transglutaminase activity and formation of SS bonding (Jiang et al., 1986; Kimura et al., 1991, Tsukamasa and Shimizu, 1990), while modori is induced by endogenous thermal stable proteases that can degrade myosin rapidly (An et al., 1994; Yongsawatdigul et al., 1995; Jiang et al., 1996, 1997; Wang and Xiong, 1998).

Disintegration of surimi gels is considered to be due to the proteases that are active at temperatures $\geq$50°C and can rapidly degrade myofibrillar proteins, particularly myosin (Wasson et al., 1992a; Wang and Xiong, 1998; Boye and Lanier 1988; Morrissey et al., 1993). Among numerous proteases presented in muscle, endo-proteolytic cysteine proteases have the most serious effect on the texture due to their thermostability and the ability to cleave the internal peptide bonds, while exopeptidase hydrolyze terminal peptide bonds (Kirshke and Barrett 1987). The most active proteases in fish muscle which can soften the surimi gels vary with species, but are generally categorized into two major groups, i.e. cathepsins (Yamashita and Konagaya 1990a, 1991b; Toyohara et al., 1993; Seymour et al., 1994; Jiang et al., 1996, 1997) and heat-stable alkaline proteinases (Makino et al., 1984, 1985; Boye and Lanier 1988; Wasson et al., 1992b).

High levels of cathepsins B, H, L, and L-like have been observed in Pacific whiting and arrowtooth flounder (Wasson et al., 1992b; An et al., 1994), chum salmon during spawning migration (Yamashita and Konagaya 1990a), mackerel (Lee et al., 1993) and bovine cardiac muscle (Wang and Xiong 1998, 1999). Arrowtooth flounder softening is due to a cysteine protease with maximum proteolytic activity at 50-60°C (Greene and Babbitt 1990). When the Pacific whiting muscle was incubated at 60°C for 30 min prior to cooking at 90°C, most myosin heavy chain (MHC) was degraded, and surimi did not form a gel (Morrissey et al., 1993). The proteolysis of muscle proteins and connective tissue of fish is also induced by the infection of Myxosporea which releases proteinases into the fish muscle tissue. In Pacific whiting, these parasites have been identified as Kudoa paniformis and K. thyrsitis (Morado and Sparks 1986), and in arrowtooth, K. thyrsitis (Greene and Babbitt 1990). The degree and stage of the infection have been linked to the variation in proteolytic activity between individual fish, which consequently results in the variation in gel strength of surimi (Morrissey et al., 1995; Toyohara et al., 1993). The majority of proteolytic activity in Pacific whiting muscle is due to cathepsin L (An et al., 1994; Seymour et al., 1994), while that in mackerel is due to cathepsins B, L and L-like (Jiang et al., 1996, 1997). Cathepsin L has a high affinity for myosin and is not completely removed by leaching during surimi processing (An et al., 1994). Purified cathepsin L from Pacific whiting and mackerel consists of a single peptide with MW of 28,800, and has a temperature optimum at 55°C and 50°C, and pH optimum at 5.25-5.5 and 5.0, respectively (Lee et al., 1993; Masaki et al., 1993; Porter et al., 1993; Seymour et al., 1994). According to Lee et al. (1993), the MW, optimal temperature and pH for cathepsin L-like were 58,000, 40°C and 5.5, respectively.

Cathepsin L activity was also shown to be the major contributing factor to the softening of chum salmon muscle during spawning migration but not feeding migration (Yamashita and Konagaya 1990b). The degradation of myofibrillar proteins by purified cathepsin L observed with SDS-PAGE was identical to that of muscle stored at 4°C for 7 days (Yamashita and Konagaya 1990b). In the extensively softened muscle of chum salmon, substantial degradation of MHC into fragments with MW of 160,000, and degradation
of troponin T, C, I and myosin light chains were observed (Yamashita and Konagaya 1991a).

Heat-stable alkaline proteinase (HAP) has often been reported as responsible for textural degradation of surimi gels. Its activity has been found in muscles from a large number of fish including rainbow trout, sardine, white croaker, carp, common mackerel, cod, herring, and Atlantic salmon (Makinodan et al., 1984; Stoknes et al., 1993, 1995). White croaker meat paste formed a poor elastic gel when heated around 60°C, while purified actomyosin from croaker muscle did not (Makinodan et al., 1985). Alkaline proteinase purified from white croaker was a heat-stable cysteine proteinase with temperature optimum at 60°C and pH optimum at 8.0 (Makinodan et al., 1987). It is composed of four different subunits (αβγδ) with MW ranging from 45,000 to 57,000. These complex subunits contribute to the thermostability of the enzyme (Makinodan et al., 1987). HAP was also purified from Atlantic menhaden, which showed similar characteristics as white croaker with an optimum activity at 60°C and pH 7.5-8.0 (Boye and Lanier 1988). It is characterized by its narrow range of pH and temperature optima, and usually not detected below 50°C (Makinodan and Ikeda 1977; Folco et al., 1988).

In spite of the extensive studies cited above, the following questions related to surimi must be answered: (1) how much of these proteinases are left in the surimi, (2) what is the frozen stability of these proteinases, and (3) are the cryoprotectants such as polyphosphate, sorbitol, and sucrose which are usually used in surimi manufacturing affecting these proteinases? To address these questions, research has been conducted and some of the results are summarized in the following sections.

**Changes in cathepsins B, L, L-like and calpain activities during surimi processing**

The activity of cathepsins B+L+L-like remaining in mackerel surimi after mincing, leaching, and NaCl-grinding processes, which was assayed with the synthetic peptide Z-Phe-Arg-MCA, was 6.02, 5.23 and 4.07 units/g of muscle (Jiang et al., 1997). Chang-Lee et al. (1989) reported that the protease activity of Pacific whiting muscle decreased gradually during surimi processing. However, as much as 87% of cathepsins B+L+L-like activity was left in mackerel surimi after washing treatment (Jiang et al., 1997). The results indicate that cathepsins B, L and L-like are very difficult to remove during surimi processing. According to Kinoshita et al. (1990), some softening-inducing proteinases in mackerel muscle were not easily removed by washing. Based on our data, cathepsins B, L and L-like are hypothesized to be the active proteases in mackerel surimi. However, in the case of Pacific whiting, cathepsin B activity sharply decreased after surimi process (An et al., 1994). Therefore, in surimi processing, removal of cathepsins B, L and L-like seemed to be species-dependent. Accordingly, a useful approach to inhibiting the gel softening may be to economically produce the natural inhibitor cystatin or to rupture the membrane of lysosomes without deteriorating the myofibrillar proteins for easy removal of cathepsins B, L and L-like before or during washing treatment.

After grinding with 2.5% NaCl, about 68% cathepsins B+L+L-like activity remained in the ground surimi (Jiang et al., 1996). According to Kinoshita et al. (1992), the proteolytic activity of sarcoplasmic-50°C-MIP (modori-inducing proteinases) from threadfin bream against actomyosin was not affected by the treatment with 10% NaCl. Furthermore, carp cathepsin B activity increased in the presence of 0.1-0.5 M NaCl (Hara et al., 1988). The loss of cathepsins B, L and L-like activities (about 32%) after grinding with 2.5% NaCl (Jiang et al., 1996) might be due to the denaturation of enzymes during grinding.

**Frozen stability of cathepsins B, L and L-like in surimi**

Although cathepsins B, L and L-like activities in mackerel surimi decreased progressively during frozen storage at -40°C, there was still 82% activity left after 8 weeks storage (Jiang et al., 1996). This might be because cathepsins B and L still existed in lysosomes that consequently protected them from denaturation during frozen storage. Cryoprotectants, such as sucrose, sorbitol and polyphosphates, have been used to prevent protein denaturation in frozen surimi (Noguchi 1974). These reagents showed little effect on cathepsin B and L that retained at least 83% of their activities after 8 weeks storage (Jiang et al., 1996). Although a decrease in cathepsin L-like activity was much faster than that in cathepsins B and L during 8 weeks storage at -40°C, there was still about 40% activity left. The activity of cathepsins B and L in mackerel surimi without cryoprotectants only slightly decreased during frozen storage. This result further supports the hypothesis that these proteinases are important in the gel softening of surimi (Jiang et al., 1996; Ho et al., 2000a).

**Degradation of surimi proteins by purified cathepsin B, L, and L-like**

Degradation of MHC in mackerel surimi with purified cathepsin B, L and L-like during 5 hrs incubation at 40 or 55°C was observed on SDS-PAGE, compared with those containing E-64, a cathepsins B, H, L and L-like inhibitor. Proteolysis was faster in samples with pH 6.5 than pH 7.0, and faster in samples incubated at 55°C than that at 40°C (Jiang et al., 1996). According to Jiang et al. (1996), cathepsins B, L and L-like not only degraded MHC, but also partially degraded actin at pH 6.5 or 7.0 after 5 hrs incubation at 55°C. These results suggested that the thermally unfolded globular actin might be hydrolyzed by cathepsins B, L and L-like. However, degradation of surimi proteins by cathepsins B, L and L-like could be effectively inhibited by E-64 (Jiang et al., 1996; Ho et al., 2000a). Some protease inhibitors from beef plasma (Hamann et al., 1990; Morrissey et al., 1993; Park et al., 1994; Wang and Xiong 1998, 1999), fish plasma (Toyohara et al., 1990b), potato and egg white.
Cathepsins B, L and L-like are active proteinases in many fish, such as Pacific whiting (An et al., 1994), chum salmon (Yamashita and Konagaya 1990a, b) and mackerel (Lee et al., 1993). Surimi gel softening has been attributed mostly to cathepsin L (An et al., 1994). From our studies, cathepsins B, L and L-like could not be completely washed out from the surimi and revealed myosin-degrading activity on samples incubated at 40º and 50ºC, pH 6.5 and 7.0 (Jiang et al., 1996; Ho et al., 2000a). Therefore, they might also be important in gel softening of mackerel surimi.

Gel softening by purified cathepsin B, L and L-like

The gel properties of surimi ground with purified cathepsins B, cathepsin L and L-like (5 unit/g of meat) decreased after 2 hrs incubation at 55ºC, compared to those of control (p<0.05) (Jiang et al., 1996). These results provided direct evidence to demonstrate that cathepsins B, L and L-like could induce softening in fish gels. Heat-stable alkaline proteinases (HAP) also deteriorate surimi gels (Su et al., 1981a, b). The strength of HAP-added gel decreased to about half that of the control gel (Makinodan et al., 1987). However, HAP isolated from threadfin bream had no effect on disintegration of surimi gels (Toyohara et al., 1990a, b). These phenomena suggested the great contribution of cathepsins B, L and L-like to softening of surimi gels. However, according to Jiang et al. (1996), E-64 could not completely inhibit the softening of mackerel protein gels, suggesting some factors, other than cysteine proteinases, causing gel softening existed in mackerel surimi.

Although Sp-50-MIP activity was not affected even in the presence of 10% NaCl (Kinoshita et al., 1992), HAP proteolytic activity against carp actomyosin gradually decreased with increasing concentration of NaCl (0.1–0.5M) (Iwata et al., 1974a, b). Our previous study indicated that cathepsins B, L and L-like could hydrolyze surimi protein in the presence of 0.6 M NaCl and result in gel softening of mackerel surimi (Jiang et al., 1996). If the muscle proteins are not properly stored, they will be hydrolyzed into fragments by various proteases depending on the species, storage temperature and pH of postmortem muscle. However, both intact muscle proteins and their proteolytic fragments can be gelled into different types of gels, i.e. firm network or loose (disintegrated) gel structures. The degree of softening varies with the extent of proteolysis. The intact or native muscle proteins can form firm network structure gels. However, if the well cross-linked network structural sols is not fixed at optimal time by heating at 85-100ºC, or the setting process is over the optimal time, the network structural gels will be disintegrated into softened gels. This phenomenon is usually called modori (in Japanese) or disintegration. As mentioned previously, HAP or cathepsin B, L and L-like have high activity at 50-70ºC and a substantial amount of activity is retained in frozen surimi even after NaCl-grinding. Therefore, when the surimi with native muscle proteins is set at 50-70ºC, it will easily form a soft gel due to the action of these proteinases.

Prevention of the gel-softening (Modori) by recombinant cystatin

Production of recombinant cystatins

Expression of the recombinant cystatin from E. coli

Although the human salivary cystatin SN (Bobek et al., 1994) or rat salivary cystatin S (Sharma et al., 1995) recombinant proteins carried a fusion protein of glutathione S-transferase, they were still expressed as insoluble inclusion body in E. coli. The necessity of urea renaturation and solubilization with dialysis treatments for recovering the active/soluble recombinant proteins would greatly increase running cost and also be time-consuming. Chen et al. (2000) and Tzeng et al. (2001, 2002) used pGEM-T Easy cloning vector containing the correct in-frame cystatin cDNA sequence to construct the chicken lung cystatin and mature carp ovarian cystatin expression vectors. The cDNA for cystatins were ligated with pET-23a(+) expression vector in Nde I and Xho I restriction enzyme sites and introduced in frame to downstream of T7 promoter of pET-23a(+) vector. High level of the soluble recombinant cystatins was successfully expressed in AD494(DE3)pLysS after 4 hr induction by IPTG (Chen et al., 2000; Tzeng et al., 2001, 2002).

Expression of the recombinant cystatin from Pichia pastoris

Chen et al. (2001) had ligated the cDNA for chicken cystatin with pGAPZαC expression vector in Xho I and Xba I restriction enzyme sites and introduced in frame to downstream of α factor in pGAPZαC vector. After transforming the pGAPZαC- chicken cystatin plasmid into Pichia pastoris X-33 expression host, the expression vector was integrated into the genomic DNA and the highest level of cystatin activity (about 6.33 units/mg) was obtained after two days shaking cultivation (Chen et al., 2001).

Effect of recombinant cystatins on the inhibition of gel softening of surimi

The gel strength of mackerel surimi without recombinant cystatin or with cathepsins B or L was much lower than those with recombinant chicken cystatin after 2 h incubation at 55ºC. Although the recombinant cystatin could not completely inhibit the gel softening of mackerel surimi presumably due to some proteases other than the cysteine proteinases, it still has high potential for using in improving the seafood quality (Chen et al., 2000). Obvious degradation on
myosin heavy chain and moderately on actin of surimi gels preincubated at 50°C for 15, 30, 60 and 90 min prior to the fixation (30 min heating at 100°C) occurred. However, only very minor degradation was observed on samples with recombinant cystatin (Chen et al., 2000). To further investigate the inhibitory effect of recombinant cystatin on gel softening of mackerel surimi, samples with/without recombinant cystatin were processed into surimi-based product. The breaking force of samples without recombinant cystatin decreased from 1025 g to 680 – 843 g, while that of samples with recombinant cystatin decreased to 960 – 858 g after 15 – 90 min preincubation at 50°C. Significant differences in breaking force, deformation (depth at the point of gel breaking) and gel strength between samples with and without recombinant cystatin added were observed (p<0.05).

**Prevention of gel-softening (Modori) during processing of surimi-based products by the addition of transglutaminase**

**Production of microbial transglutaminase**

Guinea-pig liver has been the sole source of commercial TGase for decades. The limited source and complicated separation procedures resulted in increase in the cost for obtaining this enzyme, which consequently makes it not possible to apply in food processing. Recently, efforts have been made to search for TGase from microorganisms (MTGase). MTGase was found in cultures of Streptover
ticillium sp., Streptomyces sp. and Streptover
ticillium ladakanum (Motoki et al., 1989; Ando et al., 1989; Tsai et al., 1996a, b; Zeng et al., 2001). Microbial transglutaminase makes it possible to achieve mass production of MTGase using cheap substrates. A number of examples of the application of MTGase in food processing have been announced. However, the potential for using MTGase in cosmetics, pharmaceutical products and medical treatment, remains uncertain for commercial reasons and communication difficulties.

Some MTGase producing microorganisms were screened using the hydroxamate assay and taxonomically classified as a variant of *Streptover
ticillium mobaraense* (Ando et al., 1989; Washizu et al., 1994). These microorganisms excreted MTGase into the culture broth. The critical property of MTGase, ability of the formation of G-L bonds among proteins demonstrated that the produced enzyme was MTGase (Nonaka et al., 1989). Motoki et al. (1989) reported that other *Streptover
ticillium* strains, such as *St. griseo
carneum*, and *St. cinnamoneum* subsp. *cinnamoneum* also have the ability to produce MTGase. It was also found in *Streptomyces* sp. (Ando et al., 1989) and *St. ladakanum* (Tsai et al., 1996a, b).

The fermentation procedure for the production of MTGase is generally the same as those mentioned microorganisms (Ando et al., 1989; Motoki et al., 1989). Glucose, sucrose, starch, glycerine and dextrin can be used as carbon source. Inorganic as well as organic nitrogen sources can be used, such as NH$_4$NO$_3$, (NH$_4$)$_2$SO$_4$, urea, NaNO$_3$, NH$_4$Cl, soya, rice, maize, wheat or wheat flour, bran, defatted soya bean, maize-steep liquid, peptone, meat extract, casein, amino acids and yeast extract. Necessary minerals and trace elements are phosphate, magnesium, potassium, iron, copper, zinc and vitamins, etc. Non-ion surfactant and antifoam can also be added if necessary. The culture is an aerobic fermentation so that aeration and agitation are necessary. The temperature for growth and product formation is between 25°C and 35°C, and the fermentation time is normally 2-4 days (Sakamoto et al., 1992). Since MTGase is excreted into the culture medium, cell disruption is unnecessary. Its purification thus proves to be rather easy. Consequently, its commercialization has been accelerated.

Examples of fermentation and purification of MTGase have been described by Ando et al. (1989) and Motoki et al. (1989). The microorganism was activated at 30°C for 2 days and then cultured at the same temperature for 3 days under aeration (10 l/min) and agitation (250 rpm). The broth had an enzyme activity ranging from 0.28 U/ml to 2.5 U/ml, dependent upon the strain used. After being separated by centrifugation at 3000 rpm, the supernatant was concentrated with an ultra-filtration and then chromatographed on Amberlite CG-50 and Blue Sepharose. The total recovery of MTGase activity was about 42%. However, recently a modified downstream process for purifying MTGase was described by Ho et al. (2000b). After the fermentation of *Streptover
ticillium ladakanum*, the broth was centrifuged and filtered. The enzyme was purified directly with a rapid and simple stepwise chromatography method with CM Sepharose CL-6B and Blue Sepharose Fast Flow. According to the authors, this method is simple, rapid and has a MTGase recovery of 81%.

**Effects on gel-softening (Modori)**

Seki et al. (1990) and Tsukamasa et al. (1993) found that endogenous fish TGase caused ‘suwari’ setting, hardening fish protein paste at low temperature through crosslinking. TGase from walleye Pollack, fish for surimi, has been purified and characterized by Kumazawa et al. (1996). There seems to be no doubt that both endogenous fish TGase and exogenous MTGase enable to improve the functionality of fish raw materials by increase in cross-linking (Seki, 1992; Jiang et al., 2000a, b). However, there are still some arguments as to whether the endogenous fish TGase is the only factor in suwari setting (Nowasad et al., 1993). Kumazawa et al. (1996) determined the G-L bond in several fish eggs, and suggested that endogenous TGase may correlate with the texture of raw and processed egg products. It seems that TGase treatment improves and maintains the texture-quality of fish products, which strictly depends on the freshness of raw materials. MTGase participated in the setting process of mackerel and hairtail surimi gels and mainly accelerated the cross-linking of myosin heavy chain (MHC) (Jiang et al., 1998). According to Jiang et al. (2004), the MHC of MTGase-contained mackerel and hairtail muscle protein (MP) decreased greatly, while the cross-linked MHC became predominant during incubation. No cross-linked
MHC was observed in samples without MTGase, suggesting that the MHC was a good substrate for MTGase.

The gel properties of mackerel surimi were much improved with the addition of MTGase (samples with 0.5 unit MTGase/g were about 1.5 - 2.5 fold of that of control). However, decrease in gel-forming ability of surimi-based products was found in samples with excess of MTGase, due to the over formation of the ε-(γ-glutamyl)-lysine bonds which consequently make the protein gels fragile (Asagami et al., 1995; Sakamoto et al., 1995; Seguro et al., 1995; Jiang et al., 1998, 2000a, b).

Effects of combination use of MTGase and recombinant cystatin on gel softening

Kumazawa et al. (1995) reported that TGase participated in the setting process of surimi gels and mainly accelerated the cross-linking of MHC. According to SDS-PAGE, MHC intensity decreased with the increase of MTGase and high molecular weight compounds were observed on the top of gels (Hsieh et al., 2002a, b). These components were considered resulting from cross-linking of MHC (Nowsad et al., 1995; Jiang et al., 2000a, b; Tsai et al., 1996). According to our previous study (Chen et al., 2001), the recombinant cystatin could improve the quality of mackerel surimi and produce high quality surimi analogues. The combination use of MTGase and recombinant cystatin substantially improved the gel forming ability of mackerel and hairtail surimi, and the effect of recombinant cystatin on the gel-forming ability of the mackerel surimi was more pronounced, when the MTGase was simultaneously added (Hsieh et al., 2002a, b). These data suggested that the addition of recombinant cystatin substantially prevented the texture softening caused by endogenous proteases, and MTGase further enhanced the gel property by the formation of cross-linking of MHC.

Conclusion

High levels of cysteine proteinase activities of cathepsins B, H, L, and L-like were observed in Pacific whiting and arrowtooth flounder (An et al., 1994; Wasson et al., 1992a, b), chum salmon during spawning migration (Yamashita and Konagaya 1990a) and mackerel (Jiang et al., 1997; Lee et al., 1993). These proteases have been considered to be the main factors that caused gel softening of surimi. Accordingly, the presence of these proteinases that found to be stable at 50 - 60°C had become a crucial problem for the surimi processing. The degradation of protein gels of surimi was substantially inhibited by the addition of recombinant cystatin. The gel properties of surimi were much improved with the addition of MTGase. When the MTGase was used simultaneously with recombinant cystatin, the gel forming ability of surimi was substantially improved (Hsieh et al., 2002a, b). As mentioned above, the recombinant cystatin could inhibit the gel softening of mackerel surimi caused by cathepsins and the MTGase could enhance the gel property by the formation of cross-linking of MHC. They have high potential for using in improving the seafood quality. The recombinant chicken cystatin had biological and physical properties comparable to the wild-type cystatin and the expression system developed thus far are useful and economical in terms of producing recombinant cystatin for industrial application.

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