รายละเอียดโครงการวิจัยย่อยที่ 2

ชื่อโครงการวิจัยย่อยที่ 2 **ผลร่วมระหว่างเจลาตินจากหนังปลาและสารเชื่อมประสารโปรตีนต่อสมบัติของเจลซูริมิ**
(Effect of fish skin gelatin in combination with protein cross-linkers on properties of surimi gels)

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บทคัดย่อ

จากการศึกษาผลของเจลาตินปลาที่ระดับต่าง ๆ (ร้อยละ 0, 5, 10, 15 และ 20 ของโปรตีน) ร่วมกับการใช้กรดแทนนิกที่ผ่านการออกซิไดซ์ (OTA) ที่ระดับความเข้มข้นต่าง ๆ (ร้อยละ 0, 0.05 และ 0.1) ต่อสมบัติเจลซูริมิปลาทรายแดง พบว่า เจลซูริมิมีค่าเจาะทะลุและระยะทางก่อนเจาะทะลุลดลงเมื่อระดับของเจลาตินปลาเพิ่มขึ้น การเติม OTA มีผลเพิ่มแรงเจาะทะลุและระยะก่อนเจาะทะลุของเจลซูริมิที่เติมเจลาตินปลาระดับร้อยละ 0-10 โดยผลของการเพิ่มความแข็งแรงเจลขึ้นกับระดับความเข้มข้นของ OTA ในทางตรงกันข้าม การเติม OTA ที่ระดับสูงขึ้นมีผลลดแรงเจาะทะลุและระยะก่อนเจาะทะลุของเจลซูริมิที่เติมเจลาตินปลาที่ระดับร้อยละ 15-20 การเติมเจลาตินมีผลลดปริมาณของเหลวบีบอัดในเจลซูริมิ ความขาวของเจลซูริมิมีค่าลดลงเมื่อระดับของเจลาตินปลาและ OTA เพิ่มขึ้น แถบโปรตีนไมโอซินเส้นหนัก (MHC) และแอกตินของเจลซูริมิลดลงเมื่อระดับของเจลาตินปลาเพิ่มขึ้น โดยมีผลจากการเจือจาง การเติม OTA ที่ระดับร้อยละ 0.1 มีผลลดปริมาณ MHC ในเจลซูริมิซึ่ง เติมเจลาตินปลาร้อยละ 5-20 เล็กน้อย

จากการศึกษาสมบัติของเจลซูริมิจากปลาทรายแดงที่เติมเจลาตินปลาที่ระดับต่างๆ กัน (ร้อยละ 0 – 20 ของโปรตีน) ร่วมกับการใช้เอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ (MTGase) ที่ระดับความเข้มข้นต่างๆ (0-1.2 ยูนิต/กรัม ซูริมิ) พบว่า แรงเจาะทะลุและระยะทางก่อนเจาะทะลุของเจลซูริมิลดลงเมื่อระดับของเจลาตินปลาเพิ่มขึ้นการเติม MTGase มีผลเพิ่มแรงเจาะทะลุและระยะทางก่อนเจาะทะลุของเจลซูริมิที่เติมเจลาตินปลาร้อยละ 0 -15 อย่างไรก็ตามการเติม MTGase ในระดับที่สูงขึ้นส่งผลให้แรงเจาะทะลุและระยะทางก่อนเจาะทะลุของเจลซูริมิที่เติมเจลาตินปลาร้อยละ 20 ลดลง การเติมเจลาตินปลามีผลลดปริมาณของเหลวบีบอัดของเจลซูริมิ แถบโปรตีนไมโอซินเส้นหนัก (MHC) และเจลาตินของเจลซูริมิลดลงเมื่อระดับของเจลาตินปลาที่เติมเพิ่มขึ้น ซึ่งเกิดจากผลของการเจือจาง เจลซูริมิที่เติมเจลาตินปลามีลักษณะหยาบและไม่สม่ำเสมอการเติมMTGaseที่ระดับ 1.2 ยูนิต/กรัมซูริมิ มีผลลดปริมาณของ MHC ของเจลซูริมิที่เติมเจลาตินเล็กน้อย

Abstract

Effect of fish gelatin at different levels (0, 5, 10, 15 and 20% protein substitution) in combination with oxidized tannic acid (OTA) at different concentrations (0, 0.05 and 0.1%) on gel properties of threadfin bream (*Nemipterus bleekeri*) surimi was investigated. Surimi gel had the decrease in both breaking force and deformation

as the levels of fish gelatin added increased (p<0.05). When OTA was incorporated, the increases in breaking force and deformation were noticeable in surimi gel added with 0-10% fish gelatin and strengthening effect was in dose-dependent manner. On the other hand, the addition of OTA at higher levels resultedin the decrease in both breaking force and deformation of surimi gel added with 15-20% fish gelatin (p<0.05). Addition of fish gelatin generally lowered the expressible moisture content of surimi gel. Whiteness of surimi gel decreased when the levels of both fish gelatin and OTA increased (p<0.05). Based on SDS-PAGE, band intensity of myosin heavy chain (MHC) and actin of surimi gel decreased with increasing fish gelatin levels, mainly due to dilution effect. The addition of 0.1% OTA decreased MHC in surimi gel containing 5-20% fish gelatin slightly.

Gel properties of threadfin bream (*Nemipterus bleekeri*) surimi added with fish gelatin at different levels (0-20% protein substitution) in combination with microbial transglutaminase (MTGase) at various concentrations (0-1.2 units g^{-1} surimi) were studied. Breaking force and deformation of surimi gel decreased when the levels of fish gelatin increased (p<0.05). When MTGase was incorporated, the increases in breaking force and deformation were obtained in surimi gel added with 0-15% fish gelatin. Nevertheless, the addition of MTGase at higher levels led to the decrease in both breaking force and deformation of surimi gel containing 20% fish gelatin (p<0.05). Addition of fish gelatin could lower the expressible moisture content of surimi gel. Band intensity of myosin heavy chain (MHC) and actin of surimi gel decreased when fish gelatin levels increased, mainly due to dilution effect. The coarser and irregular gel structure was obtained when fish gelatin was added. The addition of 1.2 units MTGase g^{-1} surimi decreased MHC in surimi gel containing fish gelatin slightly.

Chapter 1

Introduction and Literature Review

General Introduction

Surimi products have been widely popular for consumers. Currently, Thailand has processed surimi products for export as the second in the world after the United States. The main markets are Japan and the European Union. Export value of surimi and surimi products of Thailand was approximately 10,000 million in 2006 (NCGEB, 2007). Surimi is minced fish meat products, which has been extracted with water to remove fat and water-soluble proteins and undesirable muscle components such as blood and pigments. This process leads to the concentrated desirable myofibrillar proteins, which directly contribute to gelation (Iwata *et al.*, 2000). Surimi can be used as the raw material for many products with unique texture, such as sausage, balls,

imitation crab meat, etc. (NFI, 2006). Surimi with high quality should yield the flexible gel with white color. To improve the properties of surimi gel, a number of additives have been used, such as additives to retard the proteolysis as well as protein cross-linkers such as microbial transglutaminase (Benjakul and Visessanguan, 2003) or oxidized phenolic compound (Balange and Benjakul, 2009a; 2009b). However, those improving methods more likely result in the hardening of the gel and loss in elasticity. The use of fish gelatin might reduce the rigidity of surimi gel added with those protein cross-linkers.

Gelatin is a protein derived from collagen, which is the major structural protein in connective tissue of animal such as skin and bond and it is an important functional biopolymer that has a broad applications in many food industries (Rahman *et al.*, 2008). In addition, gelatin produced from porcine skins or bones cannot be used for some foods due to esthetic and religious objections (Sadowska *et al.*, 2003). Furthermore, the increases in consumer acceptable for kosher and halal foods have gained a demand for fish gelatin (Hou and Regenstein, 2006). Generally physicochemical properties of gelatin were affected by extraction methods (Eysturskar *et al.*, 2009), chemicals used for pretreatment as well as extraction condition (Koodziejska *et al.*, 2004). Intrinsic properties, including the chemical composition, the amino acid sequence as well as the chain length determined properties of gelatin (Badii and Howell, 2006). Recently, fish gelatin has been used to mix with surimi gel and it was found that the addition of gelatin resulted in the decrease in gel strength (Hernandez-Briones *et al.*, 2009). However, a little information regarding the use of gelatin from tropical fish as the texture modifier in surimi gel has been reported. The addition of fish gelatin along with the use of protein cross-linkers could be a means to modify the texture of surimi gel, which can fit the demand of consumers. Gelatin from the the skin of unicorn leatherjacket, by products from fish fillet processing plant, can be used as texture modifier in surimi gel. The information gained will be useful for surimi and fish processing industries in which gel properties of surimi can be improve and skin of unicorn leatherjacket (*Aluterus monoceros*) can be utilized.

Surimi has gelling property, varying dependent upon species and many factors, especially bondingings involved in protein interaction during gel formation. Additionally the ingredients have been reported to affect the textural properties of suirmi gel. To modify the textural property, especially the elasticity of resulting gel, the incorporation of gelatin can be a means to increase the elasticity via the introduction of hydrogen bonds. However, the excessive amount of gelatin can show the interfering effect on gelation of myofibriallr proteins. The addition of effective protein cross-linker along with gelatin, may be an approach to bring about the desirable textural property of

surimi gel. The appropriate pretreatment using phosphoric acid can be a means to imprive the gelling property of fish gelatin. Therefore, the better utilization of fish gelatin can be achieved, especially as the protein additive in surimi.

Literature Review

1. Surimi

Surimi is minced fish, which has been extracted with water to remove strong flavoring compounds, pigments, and nonfunctional proteins and subsequently dewatered to reduce the moisture content to approximately that of intact fish muscle (Suzuki, 1981). When fish flesh is separated from bones and skin (usually mechanically), it is called “minced fish” According to Pigott and Tucker (1990), after the minced fish is water-washed to remove fat and water-soluble components, it becomes “raw surimi”. This raw surimi is a wet concentrate of myofibrillar proteins and possesses enhanced gel-forming, water-holding, fat-binding, and other functional properties relative to minced fish (Okada, 1992).

2. Gelation of surimi

Gel forming ability of frozen surimi is the most important functional requirement of imposing good quality of surimi-based products (Saeki *et al.*, 1995). 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 Silorski, 1976). Differences in cross-linking of MHC contribute to the differences in gel-forming ability among the muscles of various fish (Benjakul *et al.*, 2007). Protein gels are three-dimensional matrixes or network, in which water is entrapped (Pomeranz, 1991). Gelation of proteins involves two steps as follows:

1. Protein denaturation: Additions of salt in combination of heating are two major factors involved in denaturation and gelation of muscle proteins. 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).

2. Aggregation: Denatured proteins begin to interact noncovalently to form a fine elastic network when surimi sol is subjected to heating process. Samejima *et al.* (1981) proposed the heat-induced gelation of myosin. It consists of two reactions as follows: 1) aggregation of the globular head segments of the myosin molecule, which is closely associated with the oxidation of sulfhydryl groups and 2) network formation resulting from the unfolding of the helical tail segment. The head portions also

associate to form “super-junctions’ which provide extra cross-linking to the gel network. The factors determining the number and kind of interactions or bonds include not only the species from which the surimi is derived (Shimizu, 1985; Suzuki, 1981) but also the heat conditions in which the gel is made (Akahane and Shimizu, 1990; Lee and Park, 2006; 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. Chan and Gill (1994) compared the denaturation and aggregation behaviors of cod and herring myosins. 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.*, 2006). Gill *et al.* (1992) reported that myosins from different fish species aggregated to different extents as temperature increased. Sano *et al.* (2006) found that reactive SH increased from 20°C to 50°C, suggesting that SH groups inside the actomyosin molecule emerged to the surface as a result of unfolding, thereby causing a gradual decrease in ATPase activity with increasing temperature. A rapid loss in enzyme activity was found from 40 °C to 50 °C, indicating conformational changes in active sites in actomyosin.

Calcium ions can form salt linkages between negatively charged sites on two adjacent proteins (Wan *et al.*, 1994). The addition of calcium salts to improve gelling properties of surimi may actually be more due to their effects on transglutaminase (TGase), a cross linking enzyme in the muscle than from ionic linkages between proteins. Addition of calcium salt in the surimi resulted in the increased gel strength of surimi (Benjakul *et al.*, 2004a; Ramirez *et al.*, 2003).

TGase is a transferase, having the systematic name 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, to various primary amines. When the ε-amino groups of lysine acts as acyl acceptor, it results in polymerization and inter- or intra-molecular crosslinking of proteins via formation of ε-(γ-glutamyl) lysine linkages (Ashie and Lanier, 2000). Transglutaminase (TGase) is an enzyme that catalyzes the cross linking of proteins through the formation of covalent bonds between protein molecules. This link enhances the strength (hardness and cohesiveness) of surimi gels. Seki *et al*. (1990) isolated TGase from Alaska pollock and found that it could induce the gelation of minced fish. Tsukamasa *et al*. (1990) reported that the strong gel forming ability of sardine was due to the formation of the non-disulfide bond which later was shown to be due to the action of TGase. Benjakul *et al.* (2007) reported that TGase played a role in setting of bigeye snapper surimi gel. TGase from different species determines the setting condition owing to the differences

in their optimal temperatures (Benjakul and Visessanguan, 2003). Benjakul and Visessanguan (2003) reported that setting mediated by endogenous TGase at the appropriate temperature and time contributed to an enhanced gel quality of surimi from two species of bigeye snapper. Highest setting response in *Priacanthus tayenus* and *Priacanthus macracanthus* surimi could be maximized at 40 and 25°C, respectively, corresponding to the optimum temperature of TGase activity from each species.

As the temperature is increased over 45-50°C, gel network (suwari) is partially disrupted to form a broken network (modori). This process is species dependent. The action of proteases has been found to promote this gel weakening (Benjakul and Visessanguan, 2003). To improve the setting of surimi and to strengthen the gel, microbial TGase has been widely used to induce the polymerization of proteins. Microbial TGase, which is capable of introduction covalent cross-linking between protein molecules, has become more popular for the surimi industry (Jiang *et al.*, 2000; Seguro *et al.*, 2006).

3. Use of additives for property improvement of surimi gel

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 geiling 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. Protease inhibitor is formulated with 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 (John Lin *et al.*, 2000). The addition of protease inhibitors or calcium compounds before freezing surimi is not necessary, 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, 2006).

3.1 Use of microbial transglutaminase (MTGase)

Microbial transglutaminase (MTGases) have been found in some microorganisms. Ando *et al.* (1989) isolated microorganism (*Streptoverticillium mobaraense*) that produced MTGase, which did not require calcium ions for activity. The isoelectric point of MTGase was approximately 8.9. The molecular weight of MTGase was previously determined to be 40,000 on both SDS-polyacrylamide eleetrophoresis (SDS-PAGE) and gel-permeation chromatography (Kanaji *et al.*, 1993). MTGase has a molecular weight of 38,000 and comprises 331 amino acid residues

(Kanaji *et al.*, 1993). The overall sequence data indicate that MTGase has a single cysteine residue. The molecular weight calculated from the amino acid composition (331 residues) is 37,842 (Motoki and Seguro, 1998). MTGase has been shown to be useful in strengthening surimi gels during the setting reaction (Seguro *et al.*, 2006). Tsukamasa *et al*. (1990) reported that high gel-forming ability of sardine was due to the formation of the non-disulflde bond, which was shown to be due to the action of TGase. Ca^{2+}-independent microbial TGase from *Streptoverticillium mobaraense* (Nonaka *et al.*, 1997) or from *Sircpiovcrticdliurn ladakanuin* (Tsai *et al.*, 1996) have been shown potential to increase the gel strength of fish surimi (Benjakul *et al.*, 2003).

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) (Kumazawa *et al.*, 1993; Lee and Park, 2006). An increase in non-disulfide polymerization and formation of ε-(γ-glutamyl) lysine isopeptides was found with increasing setting time and microbial TGase concentration (Tsukamasa *et al.*, 1990). Jiang *et al.* (2000) investigated the properties of surimi gels from threadfin bream and pollack surimi set at 30 or 45 °C with MTGase from *Streptoverticillium mobaraense*. The optimal amounts of MTGase and setting conditions were 0.3 unit/g surimi either at 30 °C for 90 mim or at 45 °C for 20 mim for threadfin bream. For pollack surimi, MTGase at a level of 0.2 unit/g surimi with setting at 30 °C for 60 min was found to be the optimum 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 maximum 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 maximum shear stress. Concentration of microbial TGase, temperature and time were optimized to improve the mechanical properties of surimi from silver carp. Optimal predicted properties were obtained by employing the following setting conditions: a concentration of microbial TGase of 8.8 g/kg of surimi, at 39.6 °C for 1 h. Under these conditions, a surimi from silver carp with shear stress of 146 kPa and shear strain of 1.59 was obtained. Shear stress was strongly affected by temperature and time, while shear strain was moderately affected (Ramirez *et al.*, 2000). Benjakul *et al.* (2008) studied the effect of MTGase at different levels (0 to 0.8 units/g sample) on the properties of gels from lizardfish (*Saurida undosquamis*) mince set at 25 °C for 2 h or 40 °C for 30 min prior to heating at 90 °C for 20 min. MTGase showed the gel strengthening effect on lizardfish mince, particularly when high amounts of MTGase

were used. For the gels added with MTGase at 0.8 units/g and set at 25 and 40 °C, the highest breaking force 93.1% and 90.7% was obtained, respectively.

The effect of MTGase on breaking strength and deformation of gels from MTGase-treated surimi (Surimi from Alaska pollock flesh) with and without setting at 30°C was studied by monitoring the formation of ε-(γ-glutamyl) lysine (GL) crosslink. In set gels, breaking strength and GL crosslink increased, and myosin heavy chain decreased correspondingly with MTGase concentration. These changes were smaller in gels prepared without setting. Results suggest that surimi gel could be improved through the formation of GL crosslinks by added MTGase in surimi (Sakamoto *et al.*, 2006).

Karayannakidis *et al.* (2008) studied the effect of MTGase and Ca^{2+} ions on the textural characteristics of heat induced surimi gels from sardines (*Sardina pilchardus*). Incorporation of 2% MTGase (w/w) and 0.2 % Ca^{2+} ions (w/w) in surimi significantly affected the textural characteristics of heat-induced surimi gels. Fish gels with MTGase and $CaCl_2$ added were firmer and more cohesive compared with the untreated gels. These differences in the textural properties of heat-induced surimi gels were attributed to the presence of MTGase and Ca^{2+} ions in the fish pastes. The former catalyzes the cross-linking reaction of myosin, while the latter activates endogenous transglutaminase (TGase), which also leads to the formation of covalent non-disulfide cross-links. However, MTGase-containing fish gels exhibited a more elastic texture, compared with the untreated fish pastes and those containing $CaCl_2$.

3.2 Phenolic compounds

Phenolic compounds can interact with side chain of amino acid group of proteins and can also affect protein functionality. Interaction of protein with phenolic compounds, especially in their oxidized form, leads to the formation of protein cross-links. This phenomenon affects functionality of modified protein, mainly gelation. Prigent *et al*. (2003) found that phenolic compounds can interact with proteins via non-covalent interactions and via covalent interactions. Two types of complexation mechanisms can be distinguished, monodentate and multidentate mechanisms (Haslam *et al.*, 1988). The multidentate mechanism generally requires a much lower phenolic ompound/protein molar ratio and thus a lower concentration of phenolic compound is needed. For the ‘‘monodentate’’ mechanism, a phenolic compound interacts with only one protein site and a higher concentration of phenolic compound is required. In addition, the size of the phenolic compound can decrease its conformational flexibility, which is observed to be an important parameter in protein–phenolic compound interactions (Frazier *et al.*, 2003).

Balange and Benjakul (2009b) studied the effect of oxidized phenolic compounds on the gel properties of mackerel surimi. Breaking force and deformation of gels increased as the oxidised phenolic compounds increased up to a particular level. Gels with addition of 0.40% oxidized ferulic acid (OFA) or 0.50% oxidised caffeic acid (OCF) had the increases in breaking force by 45 or 46.1% and in deformation by 12.2 or 28.1%, respectively, compared with that of the control. For gels with addition of 0.50% oxidised tannic acid (OTA) or 0.10% oxidized catechin (OCT), the breaking force was increased by 115.0 or 70.4% and deformation was increased by 27.5 or 28.4%, respectively. Nevertheless, the continuous decreases in both breaking force and deformation were noticeable when all oxidised phenolic compounds at the higher levels were added. The results revealed that oxidized phenolic compounds at the optimum concentration were effective in increasing gel strength of mackerel surimi. Kroll *et al*. (2003) reported that the interactions between phenolic compounds and proteins may lead to a decrease of protein digestibility, by blocking the substrate and/or inhibiting certain proteases. Covalent modification of proteins by phenolic oxidation products generated at alkaline pH was reported extensively (Rawel *et al.*, 2002). It was postulated that oxidised phenolic compounds might partially lower the proteolysis caused by endogenous proteinases. Cross-linked proteins were more likely less susceptible to proteolysis. This might be associated with gel strengthening in addition to enhanced protein cross-linking. Balange and Benjakul (2009c) studied the effect of added oxidised tannic acid (OTA) at different levels (0, 0.25, 0.50 and 0.75% of protein content) on the gel properties of mackerel (*Rastrelliger kanagurta*) surimi produced by alkaline washing process. OTA in the mackerel surimi at optimum level enhanced the interaction between myofibrillar proteins, which was associated with the formation of an ordered gel microstructure with finer strands. Thus, oxidised tannic acid showed the synergistic effect with alkaline washing process in improving the gel properties of mackerel surimi without any adverse effect on sensory properties. Balange and Benjakul (2009a) also reported that gels added with oxidised ferulic acid (OFA) or oxidised caffeic acid (OCF) had the increases in both breaking force and deformation when the levels added increased up to 0.20 and 0.15%, respectively. With the addition of 0.20% OFA, breaking force and deformation of gel increased by 28.98 and 38.06%, respectively, compared with that of the control. The addition of OCF at a level of 0.15% resulted in the increases in breaking force and deformation of gel by 29.78 and 38.63%, respectively, compared with that of the control.

3.3 Use of non muscle protein

3.3.1 Egg white

Egg white is an important ingredient for surimi seafood products. It is commonly used to improve gel texture (Chang-Lee and Le Lampila, 1990; Park and Morrissey, 1994), inhibit "modori" (gel-softening) as an enzyme inhibitor (Hamann *et al.*, 2006; Porter *et al.*, 1993), and improve whiteness. Aggregation of ovalbumin during heating results from hydrophobically driven protein–protein interactions. For this reason, the surimi industry uses ovalbumin to increase the gel forming ability of the myofibrillar protein concentrate (Damodaran, 1997). Campo-Deano and Tovar (2009) studied the influence of egg white protein at different proportions close to the standard concentrations (1.5, 2 and 2.5%) on the viscoelastic properties of crab sticks made from two types of surimi (Alaska pollock or Pacific whiting surimi). Increasing protein content of the surimi increased the gel strength of both types of crab sticks. The optimum egg albumen content was found to be about 1.5% for Alaska pollock and 2% for Pacific whiting surimi.

Angela *et al.* (2009) studied the effect of various types of egg white on characteristics and gelation of fish myofibrillar proteins. Three types of egg white protein including regular dried egg white (REW), special dried egg white (SEW) and liquid egg white (LEW) were used. The addition of SEW at 2% to 3% improved gel textural properties of Pacific whiting and Alaska pollock protein.

3.3.2 Soy protein isolate

Soy proteins, in the form of isolates or concentrates, are utilized in processed meats owing to the specific functionalities they are able to impart. Luo *et al.* (2004) studied the effects of soy protein isolate (SPI) on the properties of bighead carp surimi gel. SPI could decrease the development of modori in bighead carp surimi, and the effects of SPI on bighead carp surimi gel properties were dependent on the setting conditions. When the protein ratio of SPI in the surimi gel increased, the breaking force and breaking distance were decreased but higher breaking force was obtained by 10% SPI in bighead carp surimi when cooked after incubation at 50 °C for 60 min.

3.3.3 Whey protein concentrate

Whey protein concentrate (WPC) has commonly been used as a protein supplement, foam stabilizer, filler/water binder, thickening, emulsifying and gelling agents (Morr and Foegeding, 1990). It can be used to improve texture and nutritional value of a variety of foods such as sausages, meat balls and low-salt fish products (Giese, 1994; Ulu, 2004; Uresti *et al.*, 2004). Murphy *et al.* (2006) studied the hardness of whiting surimi gels from Atlantic whiting prepared using heated at 90 °C for 15 min. WPC 45 made the gels tougher (increased hardness and cohesiveness) whereas the other additives, WPI, EW and WPC 76, made the gels more brittle.

Rawdkuen and Benjakul (2008) studied the effects of WPC on autolysis and gel properties of surimi produced from tropical fish. The addition of WPC at concentrations up to 3% (w/w) increased the breaking force and deformation of kamaboko gels and improved the grade of surimi from some fish species. WPC (0-3%) showed inhibitory activity against autolysis in all surimi at both 60 and 65 °C in a concentration-dependent manner. Myosin heavy chain (MHC) of surimi was more retained in the presence of WPC. Breaking force and deformation of kamaboko gels of all surimi increased as levels of WPC increased. WPC at a level of 3% (w/w) significantly decreased the whiteness of gels.

3.3.4 Plasma protein

Plasma protein (i.e., beef plasma protein (BPP), porcine plasma protein (PPP), chicken plasma protein (CPP)) can be used in both the feed and food industries owing to its good nutritional value and excellent functional properties (Tybor *et al.*, 1975). Additionally, plasma protein has been reported to exhibit proteinase inhibitory activity and gel strengthening ability during heat-induced gelation of surimi (Benjakul and Visessanguan, 2000; Benjakul *et al.*, 2001b). Morrissey *et al*. (1993) reported that beef plasma protein showed the highest inhibitory activity in whiting surimi as compared to egg white and potato extract. Similar results were found in arrowtooth flounder surimi (Reppond and Babbitt, 1993). Furthermore found that BPP showed higher percentage of papain (a cysteine proteinase) inhibition followed by whey protein concentrate, potato powder and egg white. Porcine plasma and chicken plasma proteins have been found to inhibit the autolysis of surimi gel associated will gel weakening. Benjakul and Visessanguan (2000) found that porcine plasma protein showed the highest inhibitory activity in Pacific whiting surimi as compared to egg white and beef plasma protein. Proteinase inhibitor from pig plasma was found to have a molecular weight (MW) of 60-63 kDa by inhibitory activity staining with both papain and trypsin. Rawdkuen *et al.* (2004) studied the effects of chicken plasma protein (CPP) on the hydrolysis of myofibrillar protein by endogenous proteinases in surimi made from bigeye snapper and lizardfish. CPP at a level of 2% (w/w) showed the highest inhibitory activity toward sarcoplasmic proteinases and autolysis of mince and washed mince from both bigeye snapper and lizardfish. The increased breaking force and deformation of surimi gel with higher water holding capacity were obtained when the CPP concentration increased, but the higher amount of CPP added resulted in the decrease in whiteness.

3.3.5 Gelatin

Recently, fish gelation has been used as an additive in surimi gel. Hernandez-Briones *et al.* (2009) studied the effect of fish gelatin on the mechanical and functional

properties of gels. Addtion of fish gelatin into grade A surimi gave gels without the changes in their mechanical properties but WHC was improved by adding 7.5 g/kg of fish gelatin. For grade FA surimi, the mechanical properties were decreased by increasing the amount of fish gelatin. Shear stress decreased significantly when gelatin at a level of 15 g/kg was added and shear strain decreased by adding 7.5 g/kg. The fish gelatin induced only small changes in color attributes that can be detected instrumentally, but not detected by most of consumers.

4. Gelatin

Gelatin is a fibrous protein extracted from collagen and is widely used in food, material, pharmacy and photography, especially in the food and pharmaceutical industries due to its unique chemical and physical properties (Jamilah and Harvinder, 2002; Rahman *et al.*, 2008). Gelatin is widely used in foods to improve elasticity, consistency and stability. It not only can be obtained from the skin and bones of terrestrial animals, but also from fish skin or bones (Zhou and Regenstein, 2006).

4.1 Conversion of collagen to gelatin

During the collagen to gelatin transition, many noncovalent bonds are broken along with some covalent inter-and intramolecular bonds (Schiff base and aldo condensation bonds) and a few peptide bonds. This results in conversion of the helical collagen structure to a more amorphous form, known as gelatin. These changes constitute denaturation of the collagen molecule but not to the point of a completely unstructured product. If the latter event happens, glue instead of gelatin is produced (Foegeding *et al.*, 1996). Conversion of collagen into gelatin involves three steps involving 1) pretreatment processes, the removal of noncollagenous components from the skin, 2) extraction process, the conversion of collagen to gelatin by heating in the presence of water, 3) recovery of gelatin in the final form (Foegeding *et al.*, 1996; Johnston-Banks, 1990).

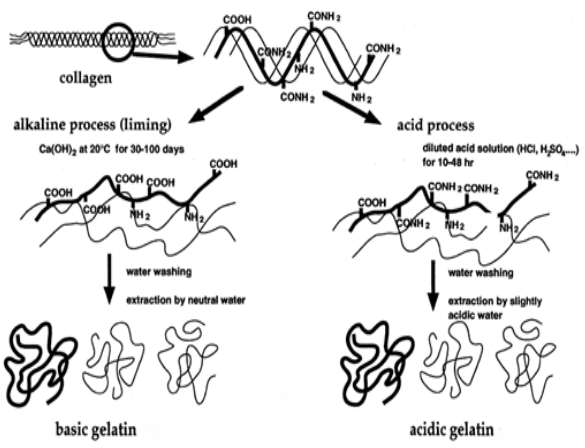

Figure 1 Preparative process for acidic and basic gelatins from collagen.
Source: Modified from Tabata and Ikada (1998)

Depending on the method in which the collagens are pretreated, two different types of gelatin (each with differing characteristics) can be produced (Fig. 1). Type A gelatin is produced from acid-treated collagen, and type B gelatin is prepared from alkali-treated collagen. Acidic treatment is most suitable for the less covalently crosslinked collagens found in pig or fish skins, while alkaline treatment is appropriate for the more complex collagens found in bovine hides (Tabata and Ikada, 1998).

The conversion of pretreated raw material into gelatin takes place in five basic stages: (I) washing, (II) extraction, (III) purification, (IV) concentration and (V) drying (Johnston-Banks, 1990). The extraction process is designed to obtain the maximum yield with the most desirable physical properties. The pH of extraction can be selected either for the maximum extraction rate (low pH) or for the maximum physical properties (neutral pH). To extract older collagens at neutral pH, a substantial proportion of the cross-links need to be cleaved, necessitating a longer liming pretreatment. If shorter liming times are used, then a lower extraction pH is necessary in order to achieve acceptable conversion rates. However, owing to the acidity present, the resultant gelatins will have lower viscosities (lower molecular weight) than those extracted at neutral pH. More efficient pretreatment conditions also allow the manufacturer to use lower extraction temperatures, resulting in gelatins of greater gel strength (bloom). Shorter treatments generally require higher extraction temperatures if neutral pH levels are chosen, resulting in gelatins of lower gel strength (Johnston-Banks, 1990). Following extraction, the gelatins are filtered to remove suspended insolubles such as fat or unextracted collagen fibres. This is usually performed using materials such as diatomaceous earth to give solutions of high clarity. The final stage is evaporation, sterilization and drying. These are performed as quickly as possible to minimize loss of properties (Johnston-Banks, 1990).

4.2 Composition of gelatin

Gelatin is a heterogeneous mixture of water-soluble proteins of high molecular weight (Budavari, 1996). On a dry weight basis, gelatin consists of 98 to 99% protein. The molecular weight of these large protein structures typically ranges between 20,000 and 250,000 (Budavari, 1996). The predominant amino acid sequence is Gly-Pro-Hyp (Poppe, 1997). As a result, gelatin contains relatively high

levels of these following amino acids: glycine (Gly) 26-34%; proline (Pro) 10-18%; and hydroxyproline (Hyp) 7-15% (Poppe, 1997). Other significant amino acids include alanine (Ala) 8-11%; arginine (Arg) 8-9%; aspartic acid (Asp) 6-7%; and glutamic acid (Glu) 10-12% (Poppe, 1997). Gelatin is not a nutritionally complete protein. It contains no tryptophan and is deficient in isoleucine, threonine, and methionine (Potter and Hotchkiss, 1998). The other sulfur-containing amino acids, cysteine and cystine, are deficient or absent. Water varies between 6 and 9% (Alais, 1991; US FDA, 1997), while ash content ranges from 0.1 to 3.25% (Veis, 1964).

Collagen encompasses all 20 amino acids. Although some differences in amino acid composition are apparent across collagens derived from different sources, there are certain features that are uniquely characteristic of all collagens. It is the only mammalian protein, which contains the large amounts of hydroxyproline and hydroxylysine, and the total imino acid (proline and hydroxyproline) content is high. Table 1 shows the typical amino acid composition of porcine gelatin from different sources (Karim and Bhat, 2009).

Table 1 Amino acid content in some fish gelatins compared to pork gelatin (residues/1000 total amino acid residues)

Amino acids	Cod skin	Alaska pollock skin	Hake	Megrim	Tilapia skin	Pork skin
Ala	96	108	119	123	123	112
Arg	56	51	54	54	47	49
Asx	52	51	49	48	48	46
Cys	0	0	-	-	0	0
Glx	78	74	74	72	69	72
Gly	344	358	331	350	347	330
His	8	8	10	8	6	4
Hyl	6	6	5	5	8	6
Ile	11	11	9	8	8	10
Leu	22	20	23	21	23	24
Lys	29	26	28	27	25	27
Met	17	16	15	13	9	4
Phe	16	12	15	14	13	14
Pro	106	95	114	115	119	132
Ser	64	63	49	41	35	35
Thr	25	25	22	20	24	18
Trp	0	0	-	-	0	0
Tyr	3	3	4	3	2	3
Val	18	18	19	18	15	26
Imino acid	156	150	173	175	198	223

Source: Karim and Bhat (2009)

4.3 Amphoteric behavior/isoelectric point of gelatin

The isoelectric point of gelatin is dependent on the type of pretreatment applied during manufacture. Type A or acid-processed gelatins have isoelectric points that can vary from 6.5 to 9.0, Type B or lime or alkaline-process gelatins have isoelectric points over a narrower pH range, typically 4.8-5.0 (Foegeding *et al.*, 1996; Johnston-Banks, 1990). Generally, acid processed gelatins have higher isoelectirc point than alkali processed gelatins (Poppe, 1997). These differences

are caused by side-reactions occurring during the pretreatment process (Johnston-Banks, 1990). If the pH of gelatin is higher than pI, it is negatively charged and if it is lower than pI, the gelatin is positively charged. In gelatin solutions of pH approx. 5.0 to 9.0, alkaline-conditioned gelatin is hence negatively charged and acid-conditioned gelatin has the positive change (Fig. 2). Below pH 5.0, all types of gelatin are thus positively charged and over pH 9.0, all are negative. Charges are then released and the molecule unfolds. This structural change influences the surface-active effect of gelatin in a positive way. The isoelectric points of collagen and gelatin bovine from skin were 8.26 and 4.88, respectively determined by Zeta potential titration (Zhang *et al.*, 2006). Net charge of gelatins from the skin of starry triggerfish (*Abalists stellatus*) pretreated with acetic acid (0.2 M) and phosphoric acid (0.2 M) became zero at pHs of 6.64-7.15 and 6.78-7.26, respectively (Ahmad and Benjakul, 2009). Muyonga *et al.* (2004) reported that the conversion of asparagines and glutamine to aspartic acid and glutamic acid during prolonged exposure of collagenous material to acid or alkali leads to the decrease in pI values.

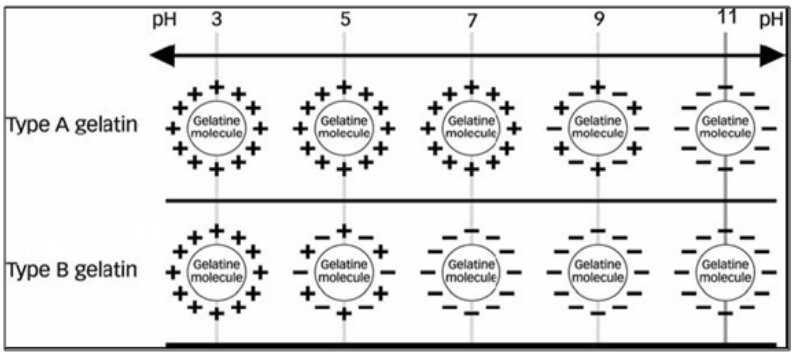

Figure 2 Charge distribution pattern of type A and B gelatins in aqueous solutions of different pH.

Source : Schrieber and Gareis (2007)

Apart from the other components, the pH of the final product is an important criterion for the selection of the optimal type of gelatin (Schrieber and Gareis, 2007).

4.4 Gelation mechanism/gel properties

All hydrodynamic properties of gelatin are dependent on its molecular weight distribution. The only difference between gel formation and viscosity is the temperature at which the system is observed. Collagen denatures at temperatures above 40 °C to a mixture of random-coil single, double and triple strands. Upon controlled cooling below the melting temperature, T_m, the reformation of the helical form occurs (Wong, 1989). The energy barrier for refolding is ~ 4 KJ/mole. The initial refolding is rapid and involves the Gly–I–I regions of

the polypeptide chain. This "nucleation" along the polypeptide chain is structurally stabilized by a certain type of water bridging. The "nucleated" polypeptide then (1) folds back into loops, with the nucleated regions aligned to form triple strands, or (2) has its nucleated region aligned with that of the other nucleated polypeptide chain. At high enough concentrations, interchain alignment becomes possible and association of polypeptide chains to form triple-helical collagen molecules can occur (Wong, 1989).

In both cases, once the nucleated regions are aligned, the remainders of the chain start renaturation. The rate of renaturation depends on the cooling temperature. Rapid cooling with large ΔT would cause rapid renaturation, resulting in areas unavailable for the formation of helical structures. As a consequence, denatured collagen with various degrees of perfection is obtained (Wong, 1989).

Gel formation, which is obtained by cooling gelatin aqueous solution after the collagen is heated, is accompanied by some characteristic changes which have been ascribed to a partial regain of collagen triple-helix structure. Gelatins from different fish species have different characteristics and properties. Gelatins from flat-fish species (sole and megrim) presented the best gelling ability and the gels were more thermostable than those from cold-adapted fish (cod and hake) (Gomez-Guillen *et al.*, 2002). This different behavior may be caused by the differences in the amino acid composition, the $\alpha 1/\alpha 2$ collagen-chain ratio, and the molecular weight distribution. Cod gelatin presented a lower alanine and imino acid content, and a decreased proline hydroxylation degree. Cod and hake gelatins had a low $\alpha 1/\alpha 2$ ratio (~1) and hake gelatin showed a highly significant decrease in β-components and other aggregates (Gomez-Guillen *et al.*, 2002).

Kittiphattanabawon *et al*. (2010) studied the characteristics of gelatin from the skins of brownbanded bamboo shark (BBS; *Chiloscyllium punctatum*) and blacktip shark (BTS; *Carcharhinus limbatus*) extracted at different temperatures (45, 60 and 75 °C) and times (6 and 12 h). Yields of gelatin from the skins of BBS and BTS were 19.06–22.81% and 21.17–24.76% (based on wet weight), respectively. Gelatins from both species extracted at 45 °C for 6 h exhibited the highest bloom strength (206–214 g), which was higher than that of commercial bovine bone gelatin (197 g). Gelatin gels from BBS skin could set at room temperature (25–26 °C) within 24 min. However, gelatin gels from BTS skin was not able to set within 3 h at the same temperature. Scanning electron microscopic study showed that gelatin gel from BBS skin presented the thicker strand than those from BTS skin and bovine bone. Cross-linked components (β-and γ-chains) and α-chains were more degraded with increasing extraction

temperatures, especially at 75 °C. Gelatin from BTS skin was more susceptible to hydrolysis than that from BBS skin.

Fernandez-Diaz *et al.*(2003) reported that gelatin from the flounder *(Platichthys flesus)* skins frozen at -12°C had lower gel strength values when compared to that from fresh skins but showed the higher melting point value. SDS-PAGE revealed that gelatin from frozen skins showed some high molecular weight aggregates and clear bands corresponding to α, β and γ -components. Gelatin from frozen skin showed less α and β-chains but more bands corresponding to lower molecular weight fragments, γ-components are less evident when both frozen at -12°C and -20°C, especially pronounced in the case of skin frozen at -12°C.

4.5 Improvement of properties of fish gelatin gel

Fish gelatin has been known to possess the low gel strength, compared to gelatin from porcine or bovine collagen (Norland, 1990). Fernandez-Diaz *et al.* (2001) studied the gel properties of two different kinds of fish gelatins prepared from cod *(Gadus rnorhua)* and hake *(Merluccius merluccius)* and modified by the coenhancers, glycerol, salt and microbial transglutaminase. Gel strength was substantially increased by the addition of coenhancers although results varied, depending on the species. For gelatin from hake skin, the highest values were obtained with 10 mg/g of transglutaminase, whereas magnesium sulphate was more effective at both concentrations (0.1 and 0.5 M) in gelatin from cod skin. Although, in both gelatins, the addition of any ingredient increased the viscosity modulus, the elastic modulus was only increased by the addition of glycerol 15% (w/v) and $MgSO_4$ 0.5 M in hake gelatins. For cod gelatin, it was increased by all ingredients. Sarabia *et al.* (2000) also examined the effects of various salts on the viscoelastic properties of a class A gelatin from megrim *(Lepi dorhombus boscii)* skins in comparison with commercial tilapia skin gelatin. Although salts generally extended the setting time of gelatins, it was found that the melting temperature was increased considerably by the addition of $MgSO_4$, $(NH)_2SO_4$, or NaH_2PO_4. Of all the salt assayed, only $MgSO_4$ improved the rheological characteristics under suitable conditions of pH and ionic strength, which differed between megrim and tilapia gelatin.

Strauss and Gibson (2004) studied the effect of plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates. Polyphenols react under oxidizing conditions with gelatin side chains and covalent cross-links are formed. Such a structure has greater mechanical strength and greater thermal stability. Rattaya *et al*. (2009) reported that the addition of seaweed extract had the impact on the property

of fish skin gelatin film. Seaweed extract can be used as the natural protein cross-linkers, which is able to modify the properties of film from gelatin or other proteins. Films incorporated with seaweed extract of *Turbinaria ornata* at pHs 9 and 10 exhibited the higher elongation at break (EAB) than the control film. However, no differences in tensile strength (TS) and transparency between films without and with seaweed extract were observed. This was associated with the formation of non-disulfide covalent bond in the film matrix, most likely induced by the interaction between oxidized phenols in seaweed extract and gelatin molecules.

Jongjareonrak *et al.* (2006) found that the addition of microbial transglutaminase (MTGase) at concentrations up to 0.005% and 0.01% (w/v) in gelatin from the skin of bigeye snapper and brownstripe red snapper resulted in the increased bloom strength of gelatin gel. However, the bloom strength of skin gelatin gel from both fish species decreased with further increase in MTGase concentration. SDS-PAGE of gelatin gel added with MTGase showed the decrease in band intensity of protein components, especially, β- and γ- components, suggesting the cross-linking of these components induced by MTGase. The addition of minor amounts of relatively low quality gelatin to whey protein improves the strength and stability of gels formed by the action of MTGase in a reducing environment (Hernandez-Balada *et al*., 2009).

Norziah *et al.* (2009) studied the effect of transglutaminase (0.5-5 mg/g gelatin) on *Tenualosa ilisha* fish gelatin in terms of melting, gelling temperature, gel strength and pH. Transglutaminase caused an increase in G′and G″ values compared to untreated gels. The results indicated that fish gelatin gel had low melting and gelling temperatures as well as gel strength even when transglutaminase was added compared to both commercial fish and commercial halal bovine gelatin gels. The modified gels obtained had higher gel strengths of 101.1 g and 90.56 g with added transglutaminase of 1.0 and 3.0 mg/g, respectively.

Research Objectives

- To investigate the effect of commercial fish skin gelatin at different levels on properties of surimi gels.
- To study the effect of commercial fish skin gelatin in combination with different protein cross-linkers on properties of surimi gels.
- To elucidate the cross-linking activity of different protein cross-linkers toward commercial fish gelatin and surimi proteins.

- To study the effect of pretreatment conditions on the properties of gelatin from skin of unicorn leatherjacket (*Aluterus monoceros*) and its impact on surimi gel properties.

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Chapter 2

Properties of Surimi Gel as Influenced by Fish Gelatin and Oxidized Tannic Acid

ABSTRACT

Effect of fish gelatin at different levels (0, 5, 10, 15 and 20% protein substitution) in combination with oxidized tannic acid (OTA) at different concentrations (0, 0.05 and 0.1%) on gel properties of threadfin bream (*Nemipterus bleekeri*) surimi was investigated. Surimi gel had the decrease in both breaking force and deformation as the levels of fish gelatin added increased ($p<0.05$). When OTA was incorporated, the increases in breaking force and deformation were noticeable in surimi gel added with 0-10% fish gelatin and strengthening effect was in dose-dependent manner. On the other hand, the addition of OTA at higher levels resultedin the decrease in both breaking force and deformation of surimi gel added with 15-20% fish gelatin ($p<0.05$). Addition of fish gelatin generally lowered the expressible moisture content of surimi gel. Whiteness of surimi gel decreased when the levels of both fish gelatin and OTA increased ($p<0.05$). Based on SDS-PAGE, band intensity of myosin heavy chain (MHC) and actin of surimi gel decreased with increasing fish gelatin levels, mainly due to dilution effect. The addition of 0.1% OTA decreased MHC in surimi gel containing 5-20% fish gelatin slightly.

Keywords: *Surimi, Gel, Fish gelatin, Oxidized tannic acid, Cross-linkers.*

1. Introduction

Surimi is minced fish meat, washed with water to remove fat and water-soluble proteins and undesirable muscle components such as blood and pigments. This process leads to the concentrated desirablemyofibrillar proteins, which directly contribute to gelation (Iwata *et al.*, 2000). Textural property of surimi is a prime factor determining the acceptability of consumer as well as market value. The properties of surimi were affected by many factors including freshness of raw material (Nolsoe *et al.*, 2011), processing (Tina *et al.*, 2010) and additives used (Julavittayanukul *et al.*, 2006; Sultanbawa and Li-Chan, 2001). Hydrocolloids, protein additives as well as microbial transglutaminase (MTGase) have been used to improve gel properties of surimi (Angela *et al.*, 2009; Benjakul and Visessanguan, 2003; Karayannakidis *et al.*, 2008; Murphy *et al.*, 2005; Sakamoto *et al.*, 2006).

Fish gelatin has been paid increasing attention as the derivative of collagen, which may play a role in improving skin/tissue properties after being ingested (Schrieber and Gareis, 2007). Gelatin, one of the most popular biopolymers, is widely used in food, pharmaceutical, cosmetic and photographic applications because of its unique functional and technological properties (Johnston-Banks, 1990; Schrieber and Gareis, 2007).In the food industry, gelatin is utilized in confections (mainly for providing chewiness, texture and foam stabilization), low-fat spreads (to provide creaminess, fat reduction and mouth feel), dairy (to provide stabilization and texturization), baked goods (to provide emulsification, gelling and stabilization) and meat products (to provide water-binding) (Johnston-Banks, 1990; Schrieber and Gareis, 2007).Since, bovine gelatin has a potential risk of spreading bovine spongiform encephalopathy (BSE), widely known as mad cow diseases and foot-and-mouth disease (FMD) (Jongjareonrak *et al.*, 2005) and porcine gelatin is prohibited by muslim and Indian (Karim and Bhat, 2009), fish gelatin has gained increasing interest as an alternative gelatin. Recently, fish gelatin has been used as the single additive in surimi and it was found to affect gel strength of surimi gel (Hernandez-Briones *et al.*, 2009). Therefore, the use of effective protein cross-linker can be a potential means to enhance gel strength of surimi gel containing gelatin, which can be cross-linked along with surimi proteins. Oxidized phenolic compound, particularly tannic acid, are able to act as protein cross-linkers, thereby strengthening gel of surimi from bigeye snapper (*Priacanthus tayenus*) (Balange and Benjakul, 2009b) and mackerel surimi (Balange and Benjakul, 2009a). The use of oxidized tannic acid (OTA) might therefore increase gel strength of surimi added with gelatin, a source of collagen derivative. However, no information regarding the effect of OTA on the properties of surimi gel added with fish gelatin has been reported.

Therefore, this study aimed to investigate textural, physical and sensory properties of surimi from threadfin beam added with fish gelatin in combination with OTA.

2. Materials and methods

2.1. Chemicals/Gelatin

Sodium dodecyl sulfate (SDS), β-mercaptoethanol (βME), glycerol, high molecular weight marker, glutaraldehyde and tannic acid (ACS reagent) were purchased from Sigma (St. Louis, Mo, U.S.A.). *N, N, N', N'*-tetramethyl ethylene diamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA). Fish gelatin was obtained from LAPI GELATINE S.p.A. (Empoli, Italy).

2.2. Preparation of OTA

OTA was prepared as per the method of Balange and Benjakul (2009b). Tannic acid was dissolved in distilled water (100 ml; 1% w/v).The solution was adjusted to pH 8 using 6 N NaOH or 6 N HCl.The prepared solution was placed in a temperature controlledwater bath (40°C) (W350, Memmert, Schwabach, Germany) and subjected to oxygenation for 1 hby bubblingthe solution with oxygen with the purity of 99.5–100% (TTS GasAgency, Hat Yai, Songkhla, Thailand) to convert tannic acid toquinone. The solution was adjusted to pH 7 by using 6 N HCl andreferred to as 'oxidized tannic acid' (OTA).

2.3. Preparation of surimi gel added with fish gelatin and OTA

Frozen surimi from threadfin bream (*Nemipterus bleekeri*)was purchased from Pacific Fish Processing Co., Ltd., Songkhla, Thailand. Surimi was then cut into small pieces and mixed with 2.5% salt in a mixer (MK-5087M, Panasonic Manufacturing Malaysia Berhad, Selangor, Malaysia). During chopping, the temperature was maintained below 10°C. Fish gelatin was then added into surimi paste at different levels (5, 10, 15 and 20% protein substitution) and chopped for 5 min. To the mixture, OTA (0.0, 0.05 and 0.1% of protein content) was then added and chopped for another 5 min. The moisture content of the mixture was adjusted to 85% with iced water. Thereafter, the mixture was chopped for another 3 min and the paste was stuffed into casing with a diameter of 2.5 cm. Both ends of the casing were sealed tightly. The paste samples were subjected to setting at 4°C for 24 h, followed by heating at 90°C for 20 min. Gel samples were cooled rapidly in iced water and kept at 4°C overnight prior to analyses.

2.4. Determination of properties of surimi gel

2.4.1. Breaking force and deformation

Breaking force (gel strength) and deformation (elasticity/deformability) of gel samples were determined using a Model TA-XT2 texture analyzer (Stable Micro System, UK) following the method of Benjakul *et al.* (2007). Gels were equilibrated at room temperature (28-30°C) for 1 h before analyzes. Five cylindrical samples (2.5 cm in diameter) were cut into the length of 2.5 cm. A spherical probe with a diameter of 0.005 m was pressed into the cut surface of a gel specimen perpendicularly at a constant depression speed (60 mm/min) until the puncture occurred.

2.4.2. Texture profile analysis

Textural profile analysis (TPA) of surimi gels was carried out usinga ModelTA-XT2 texture analyzer (Stable Micro System, Surrey, UK) (Bourne, 1978) and using a cylinder probe with a diameter of 2.5cm. Hardness, springiness, cohesiveness, gumminess and chewiness were determined.

2.4.3. Expressible moisture content

Expressible moisture content of gel samples was measured according to the method of Benjakul *et al.* (2007). Cylindrical gel samples were cut into a thickness of 5 mm (approximately 3-4 g), weighed accurately (*X*) and placed between three pieces of Whatman paper No.1 at the bottom and two piece on the top of the sample. The standard weight (5 kg) was placed on the top and hold for 2 min. The samples were then removed from the papers and weighed again (*Y*). Expressible moisture content was calculated with the following equation:

$$\text{Expressible moisture content (\%)} = 100 \times ((X-Y)/X).$$

2.4.4. Whiteness

Whiteness of gel samples was determined as described by Benjakul *et al.* (2004) using a colorimeter (model ColorFlex, HunterLab Reston, VA, USA.). CIE L^*, a^* and b^* values were measured and whiteness was calculated using following equation:

$$\text{Whiteness} = 100-[(100-L^*)^2 + a^{*2} + b^*2]^{1/2}$$

where: L^* = lightness; a^* = redness/greenness; b^* = yellowness/blueness

2.4.5. SDS-polyacrylamide gel electrophoresis

Protein patterns of gels were analyzed under reducing condition by SDS-PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85°C were added to the sample. The mixture was

then homogenized using a homogenizer (IKA Labortechnik, Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The samples were centrifuged (MIK-RO20, Hettich Zentrifugan, Germany) at 3500 xg for 20 min. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hodgen, 1940), and then mixed with sample buffer. The samples (15 μg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid.

2.4.6. Microstructure of surimi gel

Microstructure of surimi gel samples containing 0, 5, 10, 15 and 20% fish gelatin without and with 0.1% OTA was determined using scanning electron microscope (SEM). Small pieces of gel surimi were fixed with 2.5 % glutaraldehyde in 0.2 M phosphate buffer, pH 7.2 for 2 h at room temperature. Fixed specimens were dehydrated in graded ethanol solution with serial concentrations. Samples were rinses with distilled water and critical point dried (Balzers mod. CPD 030, Balzers Process Systems, Liechtenstein) using CO_2 as transition fluid. The prepared samples were mounted on copper specimen holders, sputter-coated with gold (Balzer mod. SCD 004) and examined on a JSM 5200 scanning electron microscope (JSM 5800 LV, JEOL, Ltd., Tokyo, Japan).

2.4.7. Sensory evaluation

Surimi gels containing fish gelatin at levels of 0, 5, 10, 15 and 20% fish gelatin without and with 0.1% OTA were determined for likeness using hedonic (9-point-scale) (Meilgaard *et al.*, 1999) using 30 panelists. Surimi gel without fish gelatin and OTA was used as the control.

2.5. Statistical analysis

All experiments were run in triplicate. Completely Randomized Design(CRD) was used for the entire study. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by using Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis *was* performed using the Statistical Package for Social Sciences (SPSS 10.0 for windows: SPSS Inc. Chicago, IL).

3. Results and discussion

3.1. Breaking force and deformation of surimi gel added with fish gelatin and OTA at different levels

Breaking force and deformation of gel from threadfin bream surimi added without and with fish gelatin and OTA at various levels are depicted in Fig. 1. At the same level of OTA added, the decreases in breaking force and deformation were obtained when fish gelatin at a higher level was added (p<0.05). This was more likely due to the dilution effect of fish gelatin on myofibrillar proteins, which played a role in gel formation. This result was in agreement with Hernandez-Briones *et al.* (2009) who reported that surimi gel from Alaska pollock had the decreases in shear stress and shear stain when gelatin was added. In the absence of OTA, the marked decreases in both breaking force and deformation were found with increasing gelatin added (p<0.05). Among all samples added with fish gelatin, that containing 15% fish gelatin showed the highest breaking force and deformation (p<0.05). However, breaking force and deformation of surimi gel were much lower than the control (without gelatin). The detrimental effect on mechanical properties of surimi gels might be associated with disruptive effect of the gelatin during the formation of three-dimensional structure by myofibrillar protein interactions during the gelling phenomenon, resulting in the decreases in gel strength (Hernandez-Briones *et al.*, 2009). Moreover, fish gelatin was added as the substitution of surimi protein, thereby lowering myofibrillar protein concentration for a three-dimensional network formation. Similar results were observed when protein additives were incorporated. Addition of wheat gluten or whey protein concentration in surimi fromhorse mackerel generally reduced the firmness of surimi gel and made the gel more brittle (Chen, 2000).Murphy *et al.* (2005) reported that the addition of whey protein isolate in surimi from Atlantic whiting prepared cooking at 90°C for 15 min had an adverse effect and brittle gel was obtained. At the appropriate level, some proteinaceous additives, such as egg white (Yetim and Ockerman, 1995), casein (Baker *et al.*, 2000; Uresti *et al.*, 2004) and beef plasma-thrombin (Baker *et al.*, 2000) have been shown to improve the mechanical properties of fish and surimi gels. On the other hand, some proteins and carbohydrates such as whey protein concentrate, wheat gluten (Chen, 2000; Murphy *et al.*, 2005), alginates, xanthan and high methoxyl pectins have a disruptive effect on surimi and fish gels (Barrera *et al.*, 2002; Park, 2000).

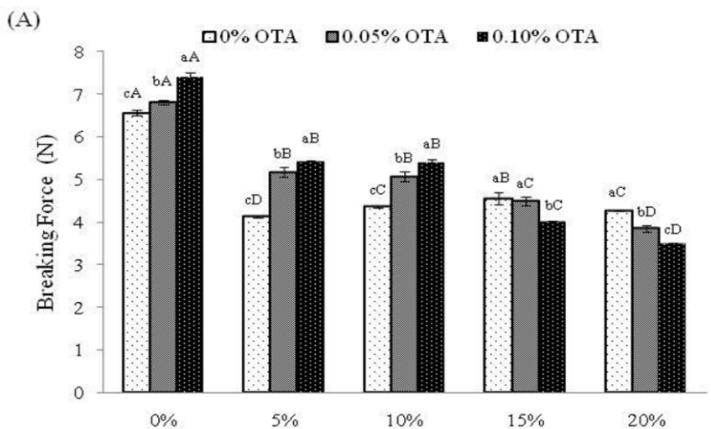

Figure 1. Breaking force (A) and deformation (B) of surimi gel added with fish gelatin and OTA at different levels. Bars represent the standard deviations (n=3). Different lowercase letters on the bars within the same level of gelatin denote the significant differences (p<0.05). Different uppercase letters on the bars within the same level of OTA denote the significant differences (p<0.05).

When OTA (0.05 and 0.1%) was incorporated in surimi, the increases in breaking force and deformation were found in sample containing 0, 5 and 10% fish gelatin (p<0.05). OTA at higher level could increase both breaking force and deformation more effectively (p<0.05). The results indicated that OTA showed the enhancing effect on surimi gel in the presence of appropriate level of fish gelatin. Balange and Benjakul (2009c) reported that 0.5 and 0.25% OTA was added to increase breaking force and deformation of surimi from mackerel (*Rastrelliger kanagurta*). OTA was found to induce cross-linking of MHC in surimi, thereby enhancing gel strength of surimi. Nevertheless, the decreases in breaking force and deformation were found in gel added with 15 and 20% fish gelatin as OTA with increasing concentrations was incorporated (p<0.05). These results indicate that OTA at higher concentration showed the detrimental effect on gel formation of surimi containing fish gelatin at levels higher than 10%. Since gelatin was not cross-linked effectively by OTA (data not shown), the remaining myofibrillar proteins more likely underwent cross-linking at the higher extent. As a result, the bundle like large aggregates could be formed. The coarser structure was generally associated with the poorer gel property (Balange and Benjakul, 2009b).

3.2. Expressible Moisture Content of Surimi Gel added with Fish Gelatin and OTA at Different Levels

Expressible moisture content of surimi gel containing the same level of OTA decreased as the amount of fish gelatin added increased (p<0.05) (Table 1). However,

there was no marked difference in expressible moisture content between sample added with 5 and 10% fish gelatin (p>0.05). No difference was also observed between sample containing15 and 20% fish gelatin (p>0.05). The result indicated that water-holding capacityof surimi gel could be improved with addition of fish gelatin. Fish gelatin is hydrophilic in nature and can bind water via H-bond. Thus, water could be hold molecularly in gel matrix containing fish gelatin. The result was in accordance with Hernandez-Briones *et al*. (2009) who reported that Alaska pollock surimi gels containing 7.5-15% of fish gelatin showed the lower expressible moisture content. It was noticed that expressible moisture content decreased when OTA at higher levels was added into surimi containing 0, 5 and 10% *($p<0.05$).* On the other hand, no changes in expressible moisture content was found in surimi gel containing 15 or 20% fish gelatin when OTA was added (p>0.05).Thus, the addition of excessive amount of OTA in surimi gel containing higher level of fish gelatin did not improve water holding capacity of surimi gel. When optimal level of OTA was added, the cross-linking of proteins could be enhanced, resulting in the formation of stronger network with a greater water holding capacity (Balange and Benjakul, 2009c).

Table 1. Expressible moisture content and whiteness of surimi gel added with fish gelatin and OTA at different levels.

Fish gelatin (%)	OTA (%)	Expressible moisture content (%)	Whiteness
0	0	2.58 ± 0.08^{aA}	81.07 ± 0.16^{aA}
	0.05	2.18 ± 0.30^{aA}	77.10 ± 0.19^{bA}
	0.1	2.14 ± 0.21^{aA}	75.61 ± 0.02^{cA}
5	0	1.87 ± 0.09^{aB}	80.24 ± 0.11^{aB}
	0.05	1.76 ± 0.04^{bBC}	76.86 ± 0.08^{bA}
	0.1	1.63 ± 0.13^{cCB}	74.79 ± 0.44^{cA}
10	0	1.86 ± 0.18^{aB}	80.49 ± 0.09^{aB}
	0.05	1.82 ± 0.25^{aBC}	77.24 ± 0.10^{bA}
	0.1	1.67 ± 0.12^{bB}	73.81 ± 0.27^{cB}
15	0	1.57 ± 0.14^{aC}	77.39 ± 0.17^{aC}
	0.05	1.58 ± 0.16^{aC}	74.43 ± 0.28^{bB}
	0.1	1.62 ± 0.06^{aB}	72.57 ± 0.11^{cC}
20	0	1.57 ± 0.07^{bC}	75.09 ± 0.27^{aD}
	0.05	1.69 ± 0.19^{aBC}	71.43 ± 0.33^{bC}
	0.1	1.70 ± 0.53^{aB}	70.23 ± 0.24^{cD}

Different lowercase superscripts in the same column within the same level of gelatin denote the significant differences (p<0.05). Different uppercase superscripts in the same column within the same level of OTA denote the significant differences (p<0.05).

3.3. Whiteness of Surimi Gel added with Fish Gelatin and OTA at Different Levels

Whiteness of all gels decreased when the levels of both fish gelatin and OTA increased (p<0.05) (Table 1). At all levels of gelatin added, the resulting gel had the decrease in whiteness as the level of OTA increased (p<0.05). When the same OTA level was incorporated, the gel had the decrease in whiteness, especially when the higher levels of gelatin were used (p<0.05). These results are in agreement with O'Connell and Fox (2011) who reported that phenolic compounds were responsible for darkening in cheese product. Similarly, the OTA decreased whiteness of gels from surimi gel from mackerel prepared by alkaline-saline washing process (Balange and Benjakul, 2009c). Balange and Benjakul (2009b) reported that the lower whiteness in surimi gels from bigeye snapper added with OTA at a level of 0.05-0.25% OTA. When tannic acid was oxidized the quinone, brown color was formed. Therefore, the additions of OTA directly lower the whiteness of surimi gel. Furthermore, fish gelatin had slightly yellowish color which might cause the decrease in whiteness of surimi gel.

OTA at level of 0.1% showed the highest efficiency in gel strengthening of surimi. Therefore, 0.1% OTA was used for further study.

3.4. Protein patterns of surimi gel added with fish gelatin at different levels with and without 0.1% OTA

Protein patterns of surimi gels without and with the addition of fish gelatin at different levels in the presence of 0.1% OTAare depicted in Fig. 2. Surimi paste contained MHC and actin as the major proteins. Decrease in MHC band intensity was found in control gel (without fish gelatin and OTA), compared with that observed in the surimi paste. The result suggested that the formation of cross-linking stabilized by non-disulphide covalent bond took place, especially during setting (Benjakul and Visessanguan, 2003). Based on the same total protein contents, the addition of fish gelatin as a substituent resulted in the dilution of muscle proteins, which was a major contributor for gel formation. This was evidenced by the lower MHC band intensity as the level of fish gelatin increased. The addition of 0.1% OTA decreased MHC in surimi gel slightly, suggesting protein cross-linking via non-disulfide covalent bonds induced by OTA. Coincidental occurrence of polymerized protein in stacking gel was noticeable.

OTA was found to induce polymerization of protein. Tannins contain sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with the proteins and other macromolecules. The interactions between phenolic compounds and proteins play a very important role in the processing of certain food products. Phenols may be oxidized easily, in an alkaline solution, to their corresponding quinones (Hurrell and Finot, 1984). The quinone, a reactive electrophilic intermediate, can readily undergo attack by nucleophiles such as lysine, methionine, cysteine and tryptophan residues in a protein chain (Hurrell and Finot, 1984). The formation of rigid molecular structures by reactions of ortho-quinones with proteins has been demonstrated by Strauss and Gibson (2004). Additionally, fish gelatin added might prevent the formation of a continuous matrix of myofibrillar proteins as indicated by the decreased breaking force and deformation (Fig. 1). The interference of myofibrillar proteins interaction was likely the major cause of the inferiority to gel strength.

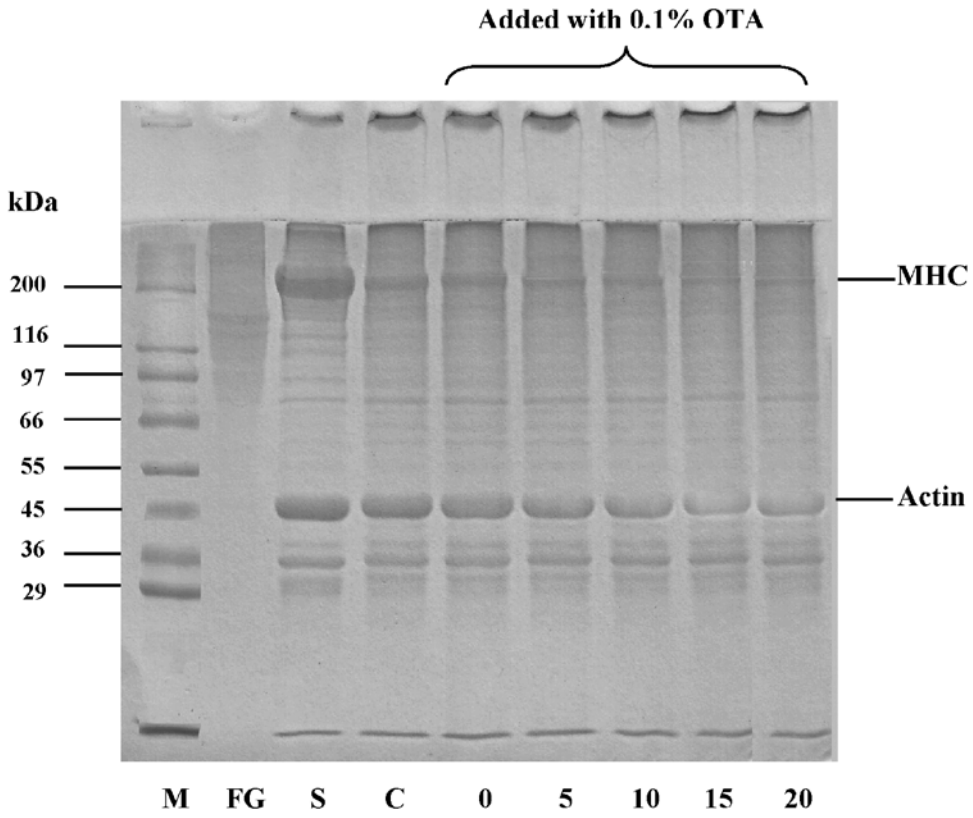

Figure 2 t
levels without and with 0.1% OTA; M: marker; FG: fish gelatin; S: surimi paste; C: control (without fish gelatin and OTA); Numbers designate the amount of fish gelatin expressed as % protein substitution of surimi.

3.5. Textural Properties of Surimi Gel added with Fish Gelatin at Different Levels with and without 0.1% OTA

TPA parameters of surimi gel added with fish gelatin and OTA at various levels are shown in Table 2. When 0.1% OTA was incorporated into surimi gel (without fish gelatin), hardness, cohesiveness, gumminess and chewiness increased ($p<0.05$). However, no change in springiness was found ($p>0.05$). This was plausibly caused by the increased protein cross-linking induced by OTA added. In the presence of 0.1% OTA, all textural parameters decreased with increasing level of fish gelatin added ($p<0.05$). The results indicated that gelatin might disturb the three-dimensional structure of myofibrillar protein networks of surimi gel. OTA could not effectively induce cross-linking of fish gelatin (data not shown) due to the constraint on amino acid composition for cross-linking. Fish gelatin contained a low content of lysine (Schrieber and Gareis, 2007). Thus, less **ε**-amino groups was available for cross-linking induced by OTA. As a consequence, OTA could not enhance gel property of surimi containing fish gelatin potentially.

Table 2. Textural properties of surimi gel added with various levels of fish gelatin and 0.1%OTA.

Samples	Hardness (N)	Springiness (cm)	Cohesiveness (ratio)	Gumminess (N)	Chewiness (N cm)
Surimi without fish gelatin and OTA	82.62±0.16^{b}	0.92±0.12^{a}	0.51±0.01^{b}	41.81±0.13^{b}	38.37±0.15^{b}
Surimi+0.1% OTA(without gelatin)	96.97±0.13^{a}	0.93±0.37^{a}	0.55±0.00^{a}	53.49±0.14^{a}	49.67±0.22^{a}
Surimi+5% fish gelatin+0.1% OTA	81.72±0.61^{b}	0.91±0.00^{a}	0.49±0.00^{b}	39.91±0.72^{b}	36.19±0.36^{b}
Surimi+10% fish gelatin+0.1% OTA	80.80±0.42^{bc}	0.89±0.01^{a}	0.48±0.00^{b}	39.90±0.22^{b}	35.79±0.11^{b}
Surimi+15% fish gelatin+0.1% OTA	75.90±0.47^{cd}	0.82±0.01^{b}	0.27±0.00^{c}	27.01±0.50^{c}	24.07±0.58^{c}
Surimi+20% fish gelatin+0.1% OTA	73.68±0.78^{d}	0.80±0.00^{b}	0.23±0.05^{c}	20.81±0.18^{d}	19.18±0.15^{d}

Values are mean ± SD (n=30). Different lowercase superscripts in the same column denote the significant difference ($p<0.05$).

3.6. Likeness Score of Surimi Gel added with Fish Gelatin at Different Levels without and with 0.1% OTA

Likeness score of surimi gels added without and with fish gelatin at various levels (0, 5, 10, 15 and 20%) and 0.1% OTA is shown in Table 3. There was no difference in likeness score for all attributes between the control surimi gel and the gel

added with 0.1% OTA (without fish gelatin addition), though the latter showed higher breaking force and deformation (Figure 1). The decrease in likeness of all attributes was observed as the level of fish gelatin increased (p<0.05). This more likely reflected the interfering effect of fish skin gelatin of surimi gel property as well as sensory property. The yellow color of fish gelatin might contribute to the lowered whiteness of resulting gel, which was in accordance with decrease in likeness of appearance. The lower texture and overall likeness score was probably due to the dilution effect of fish gelatin on myofibrillar proteins. Thus, the addition of fish gelatin adversely affected sensory property of gel from threadfin beam surimi and OTA had no pronounced gel strengthening effect on surimi containing fish gelatin.

Table 3. Likeness score of surimi gel added with various levels of fish gelatin and 0.1% OTA.

Samples	Likeness score			
	Colour	Texture	Appearance	Overall
Surimi without fish gelatin and OTA	7.46±0.18^{a}	7.80±0.13^{a}	7.36±0.10^{a}	7.53±0.10^{a}
Surimi+0.1% OTA (without gelatin)	7.33±0.11^{ab}	7.00±0.19^{ab}	7.46±0.09^{a}	7.83±0.09^{a}
Surimi+5% fish gelatin+0.1% OTA	7.16±0.11^{ab}	6.40±0.18^{b}	7.10±0.09^{ab}	6.56±0.12^{b}
Surimi+10% fish gelatin+0.1% OTA	6.80±0.11^{abc}	5.26±0.16^{c}	6.46±0.13^{b}	5.60±0.12^{c}
Surimi+15% fish gelatin+0.1% OTA	6.60±0.14^{bc}	4.23±0.17^{d}	5.46±0.16^{c}	4.50±0.16^{d}
Surimi+20% fish gelatin+0.1% OTA	6.26±0.14^{c}	3.50±0.20^{d}	4.83±0.17^{c}	4.10±0.17^{d}

Values are mean ± SD (n=30). Different lowercase superscripts in the same column denote the significant difference (p<0.05).

3.7. Microstructures of surimi gel added with fish gelatin at different levels without and with 0.1% OTA

Microstructures of surimi gels added with fish gelatin at various levels (0, 5, 10, 15 and 20%) in the presence of 0.1% OTA are illustrated in Fig. 3. Surimi gel network became finer and denser with the addition of 0.1% OTA, as compared with the control gel (without OTA). Those myofibrillar proteins could undergo the aggregation more effectively in the presence of OTA, which induced the protein cross-linking, to yield the more compact and denser gel network. The finer and more ordered structure of OTA added gel correlated with higher breaking force and deformation (Fig. 1) as well as the

lower expressible moisture content (Table 1). The microstructure of mackerel surimi with the addition of oxidized phenolics compound also had a fine matrix with small strand (Balange and Benjakul, 2009a). The coarser and irregular structure was obtained when fish gelatin was added, especially when the level added increased. Irregular structure with larger void was in agreement with poorer gel properties of surimi gel added with a higher amount of fish gelatin. This confirmed the negative impact of fish gelatin on gelation of surimi.

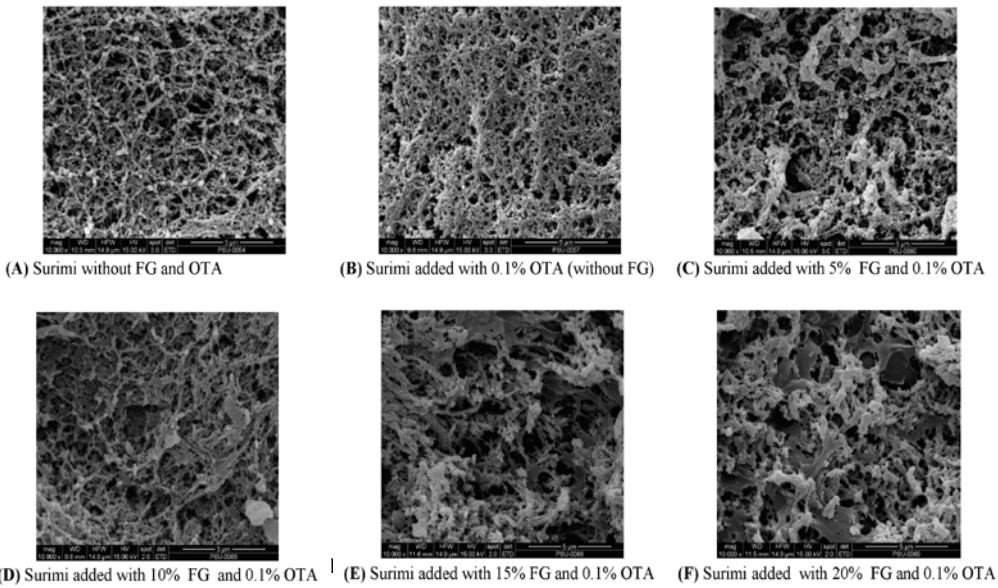

Figure 1 Microstructure of surimi gel added with fish gelatin at different levels and 0.1% oxidized tannic acid (OTA) (Magnification: 10,000 X). A: Surimi gel without FG and OTA; B: Surimi gel added with 0.1% OTA (without FG); C: Surimi gel added with 5% FG and 0.1% OTA; D: Surimi gel added with 10% FG and 0.1% OTA; E: Surimi gel added with 15% FG and 0.1% OTA; F: Surimi gel added with 20% FG and 0.1% OTA.

4. Conclusion

The addition of fish gelatin and OTA into surimi had the impact on property of surimi gel. Fish gelatin at levels of 5 or 10% was recommended to add in surimi in conjunction with 0.1% OTA to obtain surimi with grade AA. Thus, fish gelatin at an appropriate level could be used as a source of collagen derivative in surimi with satisfactory property and OTA could improve the property of surimi gel to some degree.

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Chapter 3

Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase

Abstract

Gel properties of threadfin bream (*Nemipterus bleekeri*) surimi added with fish gelatin at different levels (0-20% protein substitution) in combination with microbial transglutaminase (MTGase) at various concentrations (0-1.2 units g^{-1} surimi) were studied. Breaking force and deformation of surimi gel decreased when the levels of fish gelatin increased ($p<0.05$). When MTGase was incorporated, the increases in breaking force and deformation were obtained in surimi gel added with 0-15% fish gelatin. Nevertheless, the addition of MTGase at higher levels led to the decrease in both breaking force and deformation of surimi gel containing 20% fish gelatin ($p<0.05$). Addition of fish gelatin could lower the expressible moisture content of surimi gel. Band intensity of myosin heavy chain (MHC) and actin of surimi gel decreased when fish gelatin levels increased, mainly due to dilution effect. The coarser and irregular gel structure was obtained when fish gelatin was added. The addition of 1.2 units MTGase g^{-1} surimi decreased MHC in surimi gel containing fish gelatin slightly.

Keywords: *surim, gel, fish gelatin, microbial transglutaminase, cross-linkers.*

1. Introduction

Surimi is minced fish meat subjected to washing to remove fat, water-soluble proteins and undesirable muscle components such as blood and pigments. This process leads to the concentrated desirable myofibrillar proteins, which directly contribute to gelation (Iwata *et al*., 2001). It has been used to prepare a variety of processed foods such as kamaboko, chikuwa, fish sausages, fish balls, etc. Textural property is the prime factor determining the quality and price of surimi. Many approaches for improving the texture of surimi-based products have been therefore proposed and implemented. Protein additives have been used to improve mechanical and functional properties of fish products, e.g. egg white (Yetim and Ockerman, 1995),

casein and beef plasma-thrombin (Baker *et al*., 2000) and whey protein concentrate (WPC) (Rawdkuen and Benjakul, 2008). However, effectiveness in improving the gel quality varied with surimi. Different intrinsic factors such as level of proteases, fat content, endogenous transglutaminase, etc, play a role in determining gelation of surimi as well as efficacy in gel improvement by protein additives (Benjakul *et al*., 2008; Benjakul and Visessanguan, 2003; Benjakul *et al*., 2004b; Benjakul *et al*., 2007; Campo-Deaño and Tovar, 2009; Duangmal and Taluengphol, 2009; Rawdkuen et al., 2008). Microbial transglutaminase (MTGase) has been widely used to improve the textural quality of several foods such as ham, sausage, tofu, and noodles (Motoki and Seguro, 1998). Additionally, it has been successfully used in surimi to strengthen the gel. Benjakul et al. (2008) reported that the addition of MTGase from *Streptoverticillium mobaraense* in mince from lizardfish effectively increased breaking force and deformation of gel.

Recently, fish gelatin has been used as the single additive in surimi and it was found to lower the strength of surimi gel (Hernández-Briones *et al*., 2009). MTGase is an enzyme, which is responsible for acyl-transfer reaction between γ-carboxyamide groups of glutamine residues as ''acyl donor'' and ε-amine groups of lysine residues as ''acyl acceptor''. The reaction resulted in the formation of ε-(γ-glutamyl) lysine intra- and inter-molecular cross-links of proteins (DeJong and Koppelman, 2006). Since fish gelatin, a value-added product from fish processing byproduct, may bring about the dilution effect on myofibrillar proteins in surimi, the use of MTGase in surimi containing fish gelatin might tackle the gel weakening caused by addition of gelatin. Furthermore, MTGase might induce the cross-linking between gelatin and myofibrillar proteins. Nevertheless, no information regarding the effect of MTGase on the properties of surimi gel added with fish gelatin has been reported. Therefore, the objective of this investigation was to determine the textural, physical and sensory properties of surimi from threadfin beam added with fish gelatin in combination with MTGase.

2. Materials and methods

2.1 Chemicals/Gelatin

Sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-ME), glycerol, wide range molecular weight markers and glutaraldehyde were purchased from Sigma (St. Louis, Mo, U.S.A.). *N, N, N', N'*-tetramethyl ethylene diamine (TEMED) and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA). Fish gelatin produced from tilapia skin (~240 bloom) was obtained from LAPI GELATINE S.p.A. (Empoli, Italy). Microbial transglutaminase (MTGase) from *Streptoverticillium mobaraense* (TG-K) with actively of 100 units g^{-1} dry matter was supplied by Ajinomoto

(Thailand) Co., Ltd. (Bangkok, Thailand). This enzyme-containing product consisted of 99% maltodextrin and 1% enzyme on a mass basis.

2.2 Preparation of surimi gel added with fish gelatin and MTGase

Frozen surimi from threadfin bream (*Nemipterus bleekeri*), containing approximately 6% commercial cryoprotectants, was purchased from Pacific Fish Processing Co., Ltd., Songkhla, Thailand. Frozen surimi, having 15.2% protein as determined by the method of AOAC (2000), was partially thawed at 4°C for 8-10 h to obtain the core temperature of approximately -2°C. Surimi was then cut into small pieces and mixed with 2.5% salt in a mixer (MK-5087M, Panasonic Manufacturing Malaysia Berhad, Selangor, Malaysia). During chopping, the temperature was maintained below 10°C. Fish gelatin, containing 96.7% protein, was then added into surimi paste at different levels (5, 10, 15 and 20% protein substitution) and chopped for 5 min. To the mixture, MTGase (0.0, 0.2, 0.4 and 1.2 units g^{-1} surimi) was then added and chopped for another 5 min. The moisture content of the mixture was adjusted to 85% with iced water. Thereafter, the mixture was chopped for another 3 min and the paste was stuffed into casing with a diameter of 2.5 cm. Both ends of the casing were sealed tightly. The paste samples were subjected to setting at 4°C for 24 h, followed by heating at 90°C for 20 min. Gel samples were cooled rapidly in iced water and kept at 4°C overnight prior to analyses.

2.3 Determination of properties of surimi gel

2.3.1 Breaking force and deformation

Breaking force (gel strength) and deformation (elasticity/deformability) of gel samples were determined using a Model TA-XT2 texture analyzer (Stable Micro System, UK) as per the method of Benjakul *et al*. (2007). Gels were equilibrated at room temperature (28-30°C) for 1 h before analyses. Five cylindrical samples (2.5 cm in diameter) were cut into the length of 2.5 cm. A spherical probe with a diameter of 5 mm was pressed into the cut surface of a gel specimen perpendicularly at a constant depression speed (60 mm min^{-1}) until the puncture occurred. The force to puncture into the gel (breaking force) and the distance at which the probe punctured into the gel (deformation) were both recorded.

2.3.2 Texture profile analysis

Textural profile analysis (TPA) of surimi gels was carried out using a Model TA-XT2 texture analyzer (Stable Micro System, Surrey, UK) and using a cylinder probe with a diameter of (2.5 cm). Hardness, springiness, cohesiveness, gumminess and chewiness were determined.

2.3.3 Expressible moisture content

Expressible moisture content of gel samples was measured according to the method of Benjakul *et al*. (2007). Cylindrical gel samples were cut into a thickness of 5 mm (approximately 3-4 g), weighed accurately (X) and placed between three pieces of Whatman paper No.1 (Maidstone, Kent, England) at the bottom and two pieces on the top of the sample. The standard weight (5 kg) was placed on the top and hold for 2 min. The samples were then removed from the papers and weighed again (Y). Expressible moisture content was calculated with the following equation:

Expressible moisture content (%) = 100 × ((X-Y)/X).

2.3.4 Whiteness

Whiteness of gel samples was determined as described by Benjakul *et al.* (2004a) using a colorimeter (model ColorFlex, HunterLab Reston, VA, USA.). CIE L*, a* and b* values were measured and whiteness was calculated using the following equation:

$$\text{Whiteness} = 100\text{-}[(100\text{-}L^*)^2 + a^{*2} + b^{*2}]^{1/2}$$

where: L* = lightness; a* = redness/greenness; b* = yellowness/blueness

2.3.5 SDS-polyacrylamide gel electrophoresis

Protein patterns of surimi paste and different gels were analyzed under the reducing condition by SDS-PAGE according to the method of Laemmli (1970). To prepare the protein sample, 27 ml of 5% (w/v) SDS solution heated to 85°C were added to the sample (3 g). The mixture was then homogenized using a homogenizer (IKA Labortechnik, Selangor, Malaysia) at a speed of 11,000 rpm for 2 min. The homogenate was incubated at 85°C for 1 h to dissolve total proteins. The samples were centrifuged using a centrifuge (MIK-RO20, Hettich Zentrifugan, Germany) at 3500 ×g for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hogden, 1940) using bovine serum albumin as a standard. The sample was then mixed with sample buffer (4 ml of 10% SDS, 2 ml of glycerol, 1 ml of β-mercaptoethanol, 2.5 ml of 0.5M Tris–HCl (pH 6.8) and 0.03 g Bromophenol blue) at 1:1 ratio (v/v). The samples (15 μg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA per gel, using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After separation, the proteins were stained with 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid and destained with 50% methanol (v/v) and 7.5% (v/v) acetic acid, followed by 5% methanol (v/v) and 7.5% (v/v) acetic acid. Wide range molecular weight markers including porcine heart myosin (200 kDa), *E. coli* β-

Galactosidase (116 kDa), rabbit muscle phosphorylase B, (97 kDa), bovine serum albumin (66 kDa), bovine liver glutamic dehydrogenase (55 kDa), chicken egg ovalbumin (45 kDa), rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (36 kDa) and bovine erythrocytes carbonic anhydrase (29 kDa) were used for estimation of molecular weight of proteins.

2.3.6 Microstructure of surimi gel

Microstructure of surimi gel samples containing 0, 5, 10, 15 and 20% fish gelatin without and with the addition of 1.2 units MTGase g^{-1} surimi was determined using a scanning electron microscope. Gel samples were cut into small pieces (0.25×0.25×0.25 cm^3) and fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.2 for 2 h at room temperature. Fixed specimens were dehydrated in graded ethanol solution with serial concentrations of 50, 70, 80, 90 and 100%. Samples were rinsed with distilled water and critical point dried (Balzers mod. CPD 030, Balzers Process Systems, Liechtenstein) using CO_2 as transition fluid. The prepared samples were mounted on copper specimen holders, sputter-coated with gold (Balzer mod. SCD 004) and examined on a JSM 5200 scanning electron microscope (JSM 5800 LV, JEOL, Ltd., Tokyo, Japan).

2.3.7 Sensory evaluation

Surimi gels containing fish gelatin at levels of 0, 5, 10, 15 and 20% without and with the addition of MTGase were determined for likeness using 9-point hedonic scale (Meilgaard *et al*., 1999). Surimi gel without fish gelatin and MTGase was used as the control. Thirty panelists, who were the graduate students in Food Science and Technology program and were familiar with surimi products, were asked to evaluate for color, texture, appearance and overall likeness.

2.4 Statistical analysis

All experiments were run in triplicate. Completely Randomized Design (CRD) *was used for the entire study.* Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by using Duncan's multiple range test (Steel and Torrie, 1980). Statistical analysis *was* performed using the Statistical Package for Social Sciences (SPSS 10.0 for windows: SPSS Inc. Chicago, IL, USA.).

3. Results and discussion

3.1 Breaking force and deformation of surimi gel added with fish gelatin and MTGase at different levels

Breaking force and deformation of surimi gel from threadfin bream added with fish gelatin at different levels (0, 5, 10, 15 and 20%) in the presence of MTGase at various concentrations (0, 0.4, 0.8 and 1.2 units g^{-1} surimi) are shown in Fig. 1. Without MTGase, breaking force and deformation of surimi gel decreased (p<0.05). Among all samples added with fish gelatin in the absence of MTGase, those containing 15% fish gelatin showed the highest breaking force, whereas those added with 10% fish gelatin had the highest deformation (p<0.05). It was noted that the gels added with 5% gelatin had the lowest breaking force and deformation when MTGase was absent (p<0.05). This was more likely due to the water binding capacity of gelatin, thereby retaining more water in gel network. Gelatin is hydrophilic in nature, in which water could be imbibed effectively in gel matrix. However, gel samples added with gelatin had the lower breaking force and deformation than the control (p<0.05). This was mainly caused by the dilution effect of myofibrillar proteins in surimi as a result of gelatin addition. This result was in agreement with Hernández-Briones *et al*. (2009) who reported that surimi gel from Alaska pollock had the decreases in shear stress and shear stain when fish gelatin was added.

When MTGase was incorporated in surimi, the increases in breaking force and deformation were found in sample containing 0, 5, 10 and 15% fish gelatin (p<0.05). The increases were more intense with increasing MTGase levels (p<0.05). MTGase is able to induce the acyl transfer between acyl donors to acyl acceptor, in which ε-(γ-glutamyl) lysine linkage can be formed (Benjakul *et al*., 2008). Those non-disulfide covalent bonds contributed to the increase in gel strength (Yokoyama *et al*., 2004). For surimi added with MTGase at the same level, the decreases in breaking force and deformation were obtained when fish gelatin added increased (p<0.05). The addition of gelatin as the surimi substitutes generally led to weakness of surimi gel. This was mainly resulted from the less amounts of myofibrillar proteins in the samples. Several proteins and carbohydrates such as whey protein concentrate, wheat gluten (Chen, 2001; Murphy *et al*., 2005), alginates, xanthan and high methoxyl pectins have a disruptive effect on surimi and fish gels (Barrera *et al*., 2002; Park, 2000). The detrimental effect on mechanical properties of surimi gels might be associated with preventive effect of gelatin toward the formation of three-dimensional structure of myofibrillar proteins during the gelling process (Hernández-Briones et al., 2009). Luo *et al*. (2004) reported that when the protein ratio (10-40%) of SPI (soy protein isolate) in the surimi gel from Bighead carp (*Aristichthys nobilis*) increased, breaking force and breaking distance decreased. It was noted that MTGase at higher levels could increase

both breaking force and deformation more effectively (p<0.05), except for surimi gel containing 20% fish gelatin. Benjakul *et al.* (2008) studied the effect of MTGase at different levels (0 to 0.8 units g^{-1} surimi) on the properties of gels from lizardfish (*Saurida undosquamis*) mince set at 25°C for 2 h or 40°C for 30 min prior to heating at 90°C for 20 min. MTGase showed the gel strengthening effect on lizardfish mince, particularly when high amounts of MTGase were used. The addition of MTGase at 2 g kg^{-1} of red tilapia surimi gel gave the highest breaking force, in which the increase by 240% was obtained, compared to the control (Duangmal *et al*., 2009). The addition of MTGase is reported to induce the cross-linking of myosin heavy chain (MHC), thereby increasing the gel strength (Hsieh *et al*., 2006; Jiang *et al*., 2000; Sakamoto *et al*., 2006).

The decreases in breaking force and deformation were found in gel added with 20% fish gelatin when MTGase at high concentrations (1.2 units g^{-1} surimi) was incorporated (p<0.05). These results indicated that MTGase at higher concentrations showed the detrimental effect on gel formation of surimi containing fish gelatin at levels higher than 15%. The use of MTGase above an optimum concentration caused a detrimental effect on the textural properties of surimi gel (Lee *et al*., 2006; Ramirez *et al*., 2002). Sakamoto *et al.* (2006) suggested that the excessive formation of ε-(γ-glutamyl) lysine cross-links would inhibit a uniform development of the protein network and lower the improvement of gel strength. Gelatin was rarely cross-linked by MTGase, whereas myofibrillar protein was preferably polymerized (data not shown). This caused the disconnected gel network, leading to the weakening of result gel.

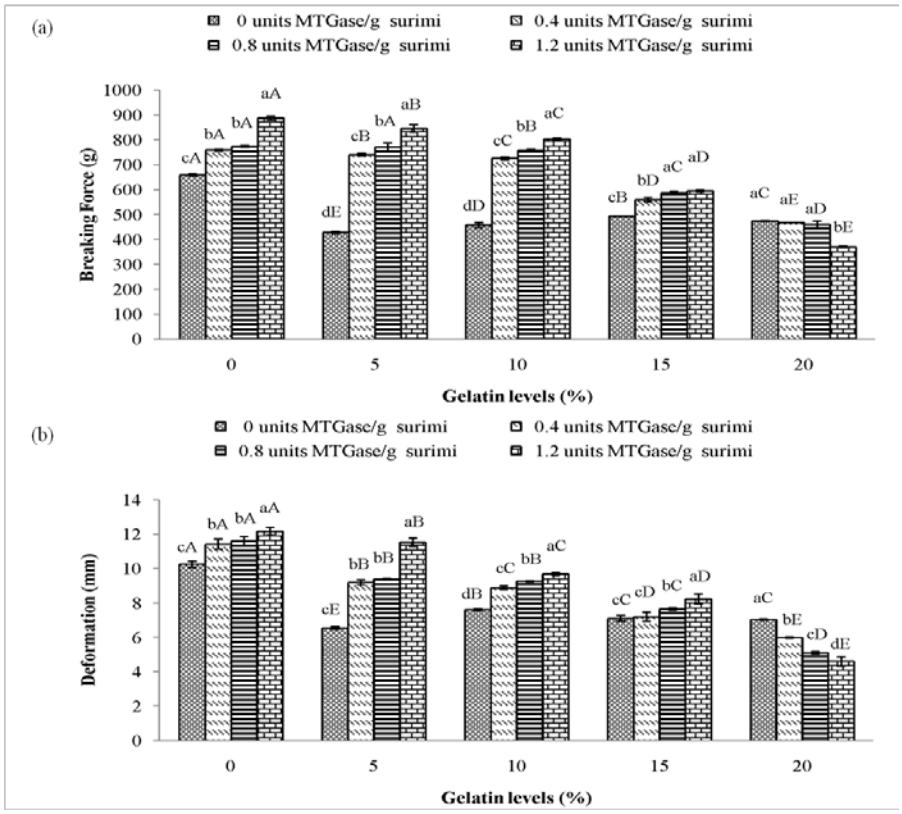

Figure 1 Breaking force (a) and deformation (b) of surimi gel added with fish gelatin and MTGase at different levels. Bars represent the standard deviations (n=3). Different lowercase letters on the bars within the same level of gelatin denote the significant differences (p<0.05). Different uppercase letters on the bar within the same level of MTGase denote the significant differences (p<0.05).

3.2 Expressible moisture content of surimi gel added with fish gelatin and MTGase at different levels

Expressible moisture content of surimi gel added with fish gelatin and MTGase at different levels is shown in Table 1. Surimi gels had the decreases in expressible moisture content when the levels of fish gelatin increased (p<0.05). The result indicated that gelatin added was able to hold water in the surimi gel effectively as evidenced by the decrease in expressible moisture content. *Fish gelatin was able to bind water via H-bond*. The result was in agreement with Hernandez-Briones *et al.* (2009) who reported that the surimi gels from Alaska pollock surimi containing 7.5-15% of fish gelatin showed an improved water holding capacity. When MTGase was incorporated in surimi, the decreased in expressible moisture content was found in sample containing 0, 5, 10 and 15% fish gelatin (p<0.05). At higher levels of MTGase, the decreases in expressible moisture content were obtained (p<0.05). Addition of

MTGase might induce the protein cross-linking, in which gel matrix could be formed and held water insides. Chanarat *et al.* (2012) found that the addition of MTGase (0-0.6 units g^{-1} surimi) in surimi from threadfin bream, Indian mackerel (*Rastrelliger kanagurta*) and sardine (*Sardinella gibbosa*) resulted in the decreases in expressible moisture content. Nevertheless, the increase in expressible moisture content was found in gel added with 20% fish gelatin when MTGase was incorporated ($p<0.05$). MTGase at higher levels plausibly induced the cross-linking of surimi proteins to a higher extent, therefore expelling gelatin from the surimi matrix. The protein network with less uniformity might not hold water effectively as indicated by the increased expressible moisture content. The increase in expressible moisture content was in accordance with the lowered breaking force and deformation (Figure. 1). The result confirmed that the use of fish gelatin along with MTGase, especially at high level, caused the negative effect on gel network formation. As a consequence, the weakened gel with pooper water holding capacity was obtained.

Table 1. Expressible moisture content and whiteness of surimi gel added with fish gelatin and MTGase at different levels.

Fish gelatin (%)	MTGase (%)	Expressible moisture content (%)	Whiteness
0	0	2.61 ± 0.28^{aA}	81.11 ± 0.07^{aA}
	0.4	2.48 ± 0.24^{aB}	81.11 ± 0.10^{aA}
	0.8	2.36 ± 0.15^{aC}	81.14 ± 0.07^{aA}
	1.2	2.14 ± 0.30^{aD}	81.12 ± 0.24^{aA}
5	0	1.99 ± 0.30^{aE}	80.56 ± 0.04^{aB}
	0.4	1.80 ± 0.15^{aF}	80.54 ± 0.13^{aB}
	0.8	1.77 ± 0.11^{aG}	80.52 ± 0.16^{aB}
	1.2	1.71 ± 0.13^{aG}	80.52 ± 0.14^{aB}
10	0	1.96 ± 0.02^{aE}	80.60 ± 0.07^{aB}
	0.4	1.80 ± 0.11^{aF}	80.51 ± 0.07^{aB}
	0.8	1.80 ± 0.26^{aF}	80.54 ± 0.24^{aB}
	1.2	1.70 ± 0.21^{cG}	80.53 ± 0.07^{aB}
15	0	1.79 ± 0.16^{aG}	79.22 ± 0.03^{aC}

	0.4	1.73±0.18^{bG}	79.16±0.01^{aC}
	0.8	1.73±0.14^{bG}	79.08±0.25^{aC}
	1.2	1.57±0.19^{cH}	79.15±0.20^{aC}
20	0	1.54±0.24^{bH}	78.58±0.09^{aE}
	0.4	1.75±0.14^{aG}	78.06±0.04^{aD}
	0.8	1.75±0.15^{aG}	78.05±0.16^{aD}
	1.2	1.76±0.20^{aG}	78.09±0.29^{aD}

Different lowercase superscripts in the same column within the same level of gelatin denote the significant differences (p<0.05). Different uppercase superscripts in the same column within the same level of MTGase denote the significant differences (p<0.05).

3.3 Whiteness of surimi gel added with fish gelatin and MTGase at different levels

Slight decreases in whiteness were found in surimi gels when fish gelatin levels increased, regardless of MTGase levels added (p<0.05) (Table 1). Fish gelatin which had slightly yellowish color more likely resulted in the decrease in whiteness of surimi gel. Whiteness of gel prepared from red tilapia (*O. niloticus* × *O. placidus*) surimi added with egg white, beef plasma protein and sodium ascorbate in combination with MTGase had the decrease in whiteness (Duangmal *et al*., 2009). Furthermore, Rawdkuen *et al.* (2004) reported that gel of surimi from lizardfish had the decreased whiteness when chicken plasma protein was added. However, there was no difference

in whiteness of surimi gels added with increasing levels of MTGase at all fish gelatin levels used ($p>0.05$).

MTGase at a level of 1.2 units g^{-1} surimi showed the highest efficiency in gel strengthening of surimi added with gelatin (0-15%). Therefore, 1.2 units MTGase g^{-1} surimi was used for further study.

3.4 Protein patterns of surimi gel added with fish gelatin at different levels with and without MTGase

Protein patterns of surimi gels without and with the addition of fish gelatin at different levels in the presence of 1.2 units MTGase g^{-1} surimi are depicted in Fig. 2. Surimi paste contained myosin heavy chain (MHC) and actin as the major proteins. Decrease in MHC band intensity was found in the control gel (without fish gelatin and MTGase), when compared with that observed in surimi paste. The decrease in band intensity of MHC indicated the formation of ε-(γ-glutamyl) lysine intra- and inter-molecular cross-links of proteins induced by endogenous TGase. Nevertheless, no marked changes in actin band were found. Endogenous TGase plays a role in setting of surimi, in which non-disulfide covalent bonds was formed (Benjakul *et al*., 2003). Cross-links were not be dissociated by the mixture of SDS and β-mercaptoethanol used for electrophoresis (DeJong et al., 2006; Jiang *et al*., 2000). The addition of MTGase (1.2 units g^{-1} surimi) decreased MHC band intensity in surimi gel to higher extent, compared with that found the in the control gel. This suggested more protein cross-linking ability via ε-(γ-glutamyl) lysine bonds by MTGase. The improvement of gel strength in some tropical fish was reported to be achieved by setting at 40°C for 30 min before being heated at 90°C for 20 min. Setting was associated with the increased cross-linking of MHC and formation ε-(γ-glutamyl) lysine isopeptide (Benjakul *et al*., 2003; Benjakul *et al*., 2004b). Jiang *et al*. (2000) reported that the properties of surimi gels from threadfin bream and pollack surimi set at 30 or 45°C added with MTGase from *Streptoverticillium mobaraense* were markedly improved. MTGase played the role in the cross-linking of golden threadfin bream and pollack actomyosin as indicated by gradual decrease in MHC band. Yongsawatdigul and Piyadhammaviboon (2005) reported that protein cross-linking in lizardfish surimi was catalyzed by MTGase at 40°C for 1 h prior to heating at 90°C for 30 min. It was noted that MHC and actin bands of surimi gel added with fish gelatin decreased as the gelatin level added increased. This indicated that myofibrillar proteins were diluted by gelatin added. However, the cross-linking induced by both endogenous TGase and MTGase still occurred as indicated by polymerized protein on the stacking gel. Therefore, gel property was determined not only by cross-linking but also by the alignment of proteins in the matrix.

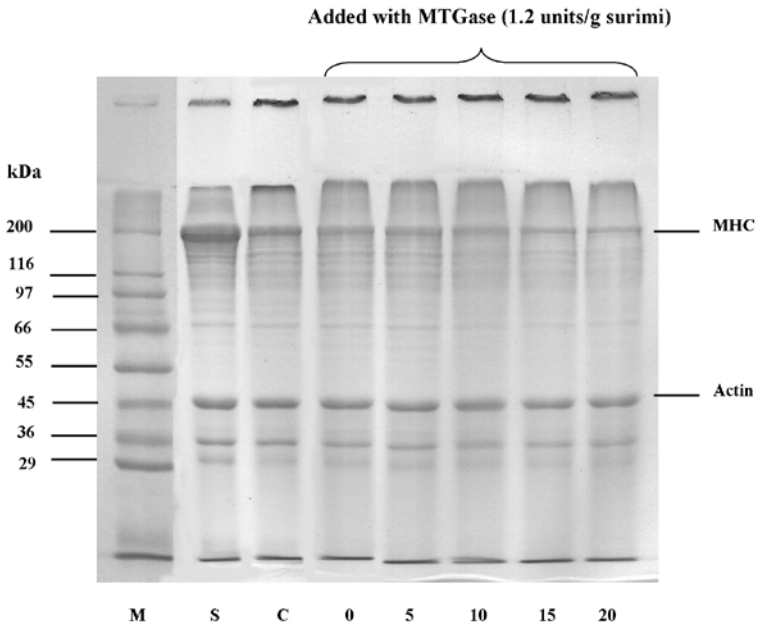

Figure 2 S ıt different levels without and with 1.2 units MTGase g^{-1} surimi. M: marker; S: surimi paste; C: control (without fish gelatin and 1.2 units MTGase g^{-1} surimi); numbers designate the amount of fish gelatin (% protein substitution of surimi).

3.5 Textural properties of surimi gel added with fish gelatin at different levels with and without MTGase

TPA parameters of surimi gel added with fish gelatin at various levels in the presence of 1.2% MTGase are shown in Table 2. When MTGase (1.2 units g^{-1} surimi) was incorporated into surimi gel (without fish gelatin), hardness, cohesiveness, gumminess and chewiness increased ($p<0.05$). However, no change in springiness was observed ($p<0.05$). This was more likely caused by the increased protein cross-linking induced by MTGase added. MTGase enhanced the strength (hardness and cohesiveness) of surimi gels (Seki *et al*., 1990). Although MTGase at a level of 1.2 units g^{-1} surimi was added, all textural parameters decreased with increasing levels of fish gelatin added ($p<0.05$). The results indicated that gelatin might disturb the formation of three-dimensional network of myofibrillar proteins, thereby exhibiting the detrimental effect on textural properties of surimi gel. MTGase could not effectively induce cross-linking of fish gelatin (data not shown) due to the constraint on amino acid composition for cross-linking. Fish gelatin contained a low content of lysine (Schrieber and Gareis, 2007). Thus, less **ε**-amino groups were available as an acyl acceptor for cross-linking reaction induced by MTGase. As a consequence, MTGase could not enhance gel property of surimi containing fish gelatin potentially as that found in surimi gel without gelatin. Generally, the addition of fish gelatin resulted in the poorer textural properties of surimi gel.

Table 2. Textural properties of surimi gel added with various levels of fish gelatin and MTGase at a level of 1.2 units g^{-1} surimi.

Samples	Hardness (N)	Springiness (cm)	Cohesiveness (ratio)	Gumminess (N)	Chewiness (N cm)
Surimi without FG and MTGase	88.49±0.03^{b}	0.96±0.10^{a}	0.53±0.01^{b}	44.36±0.10^{b}	38.37±0.03^{b}
Surimi+1.2 units MTGase g^{-1} (without FG)	98.08±0.25^{a}	0.99±0.16^{a}	0.57±0.00^{a}	57.84±0.08^{a}	49.67±0.86^{a}
Surimi+5% FG+1.2 units MTGase g^{-1}	87.53±0.42^{b}	0.95±0.01^{a}	0.51±0.02^{b}	43.65±0.09^{b}	36.19±0.51^{b}
Surimi+10% FG+1.2 units MTGase g^{-1}	86.16±0.11^{bc}	0.94±0.00^{a}	0.50±0.00^{b}	43.51±0.31^{b}	35.79±0.23^{b}
Surimi+15% FG+1.2 units MTGase g^{-1}	81.37±0.45^{cd}	0.86±0.01^{b}	0.29±0.00^{c}	31.01±0.08^{c}	24.07±0.39^{c}
Surimi+20% FG+1.2 units MTGase g^{-1}	79.03±0.08^{d}	0.84±0.01^{b}	0.25±0.00^{c}	24.25±0.42^{d}	19.18±0.07^{d}

Values are mean ± SD (n=3). Different lowercase superscripts in the same column denote the significant difference (p<0.05).

3.6 Likeness score of surimi gel added with fish gelatin at different levels with and without MTGase

Likeness score of surimi gels added without and with fish gelatin at various levels and 1.2 units MTGase g^{-1} surimi is shown in Table 3. Score of texture and overall likeness of MTGase added gel was lower than that of control (p<0.05). The addition of MTGase resulted in the rigid gel caused by the enhanced protein cross-linking. In the presence of MTGase (1.2 units g^{-1} surimi), the decrease in likeness of all attributes, except color likeness, was more pronounced as the level of fish gelatin increased (p<0.05). This more likely reflected the interfering effect of fish skin gelatin on surimi gel property as well as sensory property. Surimi gel added with only fish gelatin, especially at high levels, (without MTGase) was unacceptable (data not shown). Non-uniform appearance caused by the gelatin cluster distributed in surimi gel matrix led to the low acceptability of gel added with gelatin. Thus, the addition of fish gelatin adversely affected sensory property of gel from threadfin beam surimi and MTGase had no pronounced impact on gel of surimi containing fish gelatin.

Table 3. Likeness score of surimi gel added with various levels of fish gelatin and MTGase at a level of 1.2 units g^{-1} surimi.

Samples	Color	Texture	Appearance	Overall
Surimi without FG and MTGase	7.63±1.09^{a}	7.30±1.29^{a}	7.40±1.13^{a}	7.43±1.19^{a}
Surimi+1.2 units MTGase g^{-1} (without FG)	7.53±1.00^{a}	5.47±1.84^{c}	7.30±1.02^{a}	6.07±1.81^{c}
Surimi+5% FG+1.2 units MTGase g^{-1}	7.63±1.33^{a}	6.37±1.66^{b}	6.86±1.35^{ab}	6.67±1.00^{b}
Surimi+10% FG+1.2 units MTGase g^{-1}	7.62±1.46^{a}	6.20±1.64^{b}	6.56±1.23^{abc}	6.50±1.37^{b}
Surimi+15% FG+1.2 units MTGase g^{-1}	7.76±0.99^{a}	4.30±1.47^{d}	6.43±1.22^{bc}	4.80±1.20^{d}
Surimi+20% FG+1.2 units MTGase g^{-1}	7.73±1.16^{a}	4.17±1.03^{d}	5.83±1.19^{c}	4.76±1.11^{d}

Values are mean ± SD (n=3). Different lowercase superscripts in the same column denote the significant difference (p<0.05).

3.7 Microstructures of surimi gel added with fish gelatin at different levels with and without MTGase

Microstructures of surimi gels added with fish gelatin at various levels in the presence of 1.2 units MTGase g^{-1} surimi are illustrated in Fig. 3. Surimi gel network

became finer and denser with the addition of 0.1% MTGase, as compared with the control gel (without MTGase). Those myofibrillar proteins could undergo the cross-linking more effectively in the presence of MTGase, thereby yielding the more compact and denser gel network. The finer and more ordered structure of MTGase added gel correlated with higher breaking force and deformation (Figure 1) as well as the lowered expressible moisture content (Table 1). The coarser and irregular structure was obtained when fish gelatin was added, especially when the level of gelatin added increased. Irregular structures with larger voids were in agreement with of poorer gel properties of surimi gel added with a higher amount of fish gelatin. Network with larger voids was not strong and not resistant the force applied. Additionally, it could not hold water effectively. This confirmed the negative impact of fish gelatin on gelation of surimi.

4. Conclusion

The addition of fish gelatin up to 10% in conjunction with 1.2 units MTGase/g surimi was recommended to obtain surimi with grade AA. Thus, fish gelatin at an appropriate level could be used as a source of collagen derivative in surimi. However, gel containing gelatin had the decrease in sensory property. Future study is still needed to combat this obstacle.

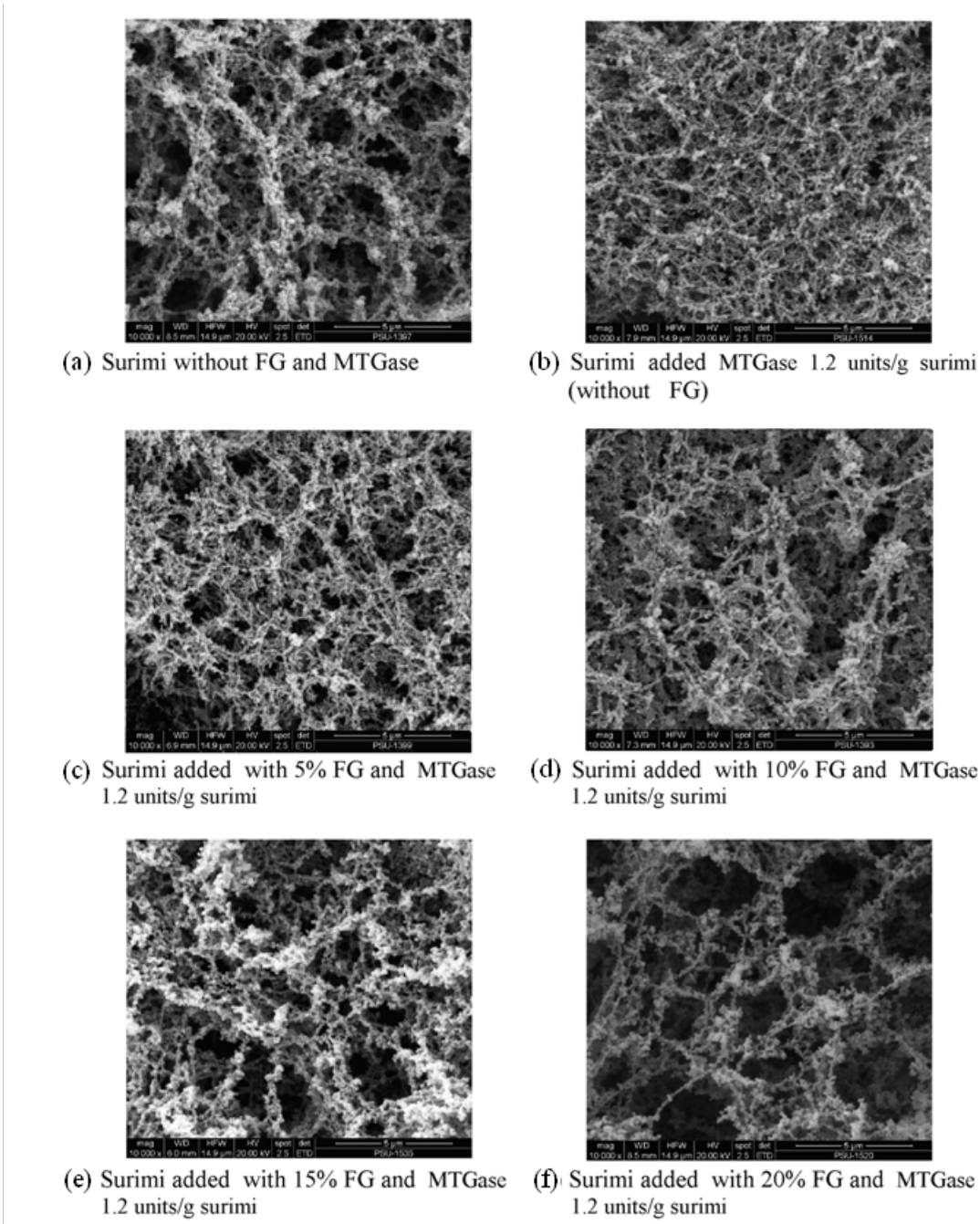

Figure 3 Electron microscopic images of surimi gel added with fish gelatin (FG) at different levels and MTGase at a level of 1.2 units g^{-1} surimi (Magnification: 10,000×). A: surimi gel without gelatin and MTGase; B: surimi gel added with MTGase 1.2 units g^{-1} surimi (without gelatin); C: surimi gel added with 5% gelatin and MTGase 1.2 units g^{-1} surimi; D: surimi gel added with 10% gelatin and MTGase 1.2 units g^{-1} surimi; E: surimi gel added with 15% gelatin and MTGase 1.2 units g^{-1} surimi; F: surimi gel added with 20% gelatin and MTGase 1.2 units g^{-1} surimi.

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