Effect of Fish Skin Gelatin in Combination with Protein Cross-Linkers on Properties of Surimi Gels

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บทคัดย่อ

จากการศึกษาสมบัติของเจลซูริมิจากปลาทรายแดง (*Nemipterus bleekeri*) ที่มีการเติมเจลาตินจากปลาทางการค้าที่ระดับความเข้มข้นต่างๆ (ทดแทนโปรตีนร้อยละ 0 5 10 15 และ 20) ร่วมกับกรดแทนนิกที่ผ่านการออกซิเดชันที่ระดับความเข้มข้นแตกต่างกัน (ร้อยละ 0 0.05 และ 0.1) พบว่าเจลซูริมิที่ได้มีค่าแรงก่อนเจาะทะลุและระยะทางก่อนการเจาะทะลุลดลง เมื่อระดับของเจลาตินเพิ่มขึ้น (p<0.05) ทั้งนี้เนื่องมาจากผลของการเจือจางปริมาณโปรตีนไมโอไฟบริล การเติมกรดแทนนิกที่ผ่านการออกซิเดชันในเจลซูริมิที่เติมเจลาตินจากปลาทางการค้าร้อยละ 0-10 ส่งผลให้ค่าแรงก่อนการเจาะทะลุและระยะก่อนการเจาะทะลุมีค่าเพิ่มขึ้นและประสิทธิภาพในการเพิ่มความแข็งแรงเจลแปรตามปริมาณของกรดแทนนิกที่ใช้ ในขณะที่การเติมกรดแทนนิกที่ผ่านการออกซิเดชันในระดับที่สูงขึ้น ส่งผลให้ค่าแรงเจาะทะลุและระยะทางก่อนการเจาะทะลุของเจลซูริมิที่ผ่านการเติมเจลาตินทางการค้าร้อยละ 15-20 มีค่าลดลง (p<0.05) การเติมเจลาตินจากปลาทางการค้าส่งผลให้ปริมาณของเหลวบีบอัดของเจลซูริมิมีค่าลดลง ค่าความขาวของเจลซูริมิลดลงเมื่อระดับของเจลาตินจากปลาทางการค้าและกรดแทนนิกที่ผ่านการออกซิเดชันเพิ่มขึ้น การเติมกรดแทนนิกที่ผ่านการออกซิเดชันร้อยละ 0.1 ในเจลซูริมิที่ผ่านการเติมเจลาตินจากปลาทางการค้าร้อยละ 5-20 มีผลให้ความเข้มข้นของแถบไมโอซินเส้นหนักลดลงเล็กน้อย

เมื่อเติมเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ที่ระดับความเข้มข้นแตกต่างกัน (0.0 0.2 0.4 และ 1.2 ยูนิต/กรัม ซูริมิ) ร่วมกับเจลาตินจากปลาทางการค้า (ร้อยละ 0-20) เจลซูริมิที่ผ่านการเติมเจลาตินจากปลาทางการค้าร้อยละ 0-15 มีค่าแรงก่อนเจาะทะลุและระยะทางก่อนเจาะทะลุเพิ่มขึ้น โดยเฉพาะอย่างยิ่งเมื่อระดับเอนไซม์ทรานส์กลูทามิเนสเพิ่มขึ้น อย่างไรก็ตามการเติมเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ในระดับที่สูงขึ้นส่งผลต่อการลดลงของค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุสำหรับเจลซูริมิที่ผ่านการเติมเจลาตินจากปลาทางการค้าร้อยละ 20 (p<0.05) นอกจากนี้การเติมเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ที่ระดับความเข้มข้น 1.2 ยูนิต/กรัม ซูริมิ ส่งผลให้ความเข้มของแถบไมโอซินเส้นหนักลดลงเล็กน้อยสำหรับเจลซูริมิที่ผ่านการเติมเจลาตินจากปลาทางการค้าที่ระดับร้อยละ 5-20 ซึ่งแสดงถึงความสามารถในการเชื่อมประสาน

จากการศึกษาผลของเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ที่ระดับ 1.2 ยูนิต/กรัม ซูริมิ ต่อสมบัติของเจลที่ผ่านการเติมเจลาตินจากวัวและเจลาตินวัวผสมเจลาตินปลา (BFGM; 1:1 2:1 1:2 4:1 และ 1:4) ทดแทนโปรตีนปลาร้อยละ 10 พบว่าการเติมเอนไซม์ทรานส์กลูทามิเนสที่ระดับ 1.2 ยูนิต/กรัม ซูริมิ ร่วมกับเจลซูริมิที่ผ่านการเติมและไม่เติมเจลาตินจากวัวร้อยละ 10 ส่งผลต่อการเพิ่มค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุ (p<0.05) นอกจากนี้ เจลซูริมิที่ผ่านการเติม เจลาตินผสม ทุกอัตราส่วนมีค่าแรงก่อนเจาะทะลุและระยะก่อนเจาะทะลุเพิ่มสูงขึ้น โดยเปรียบเทียบกับเจลซูริมิที่เติมเจลาตินจากวัว เมื่อเติมร่วมกับเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์

การเติมเจลาตินจากหนังปลาวัวที่ผ่านการสกัดที่อุณหภูมิ 50 องศาเซลเซียส ในเจลซูริมิที่ระดับความเข้มข้นทดแทนโปรตีนร้อยละ 10 เปรียบเทียบกับการเติมเจลาตินจากปลาทางการค้าพบว่า เจลซูริมิที่เติมเจลาตินทั้งสองชนิดมีค่าแรงเจาะทะลุและระยะทางก่อนเจาะทะลุลดลง เมื่อเติมเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ (1.2 ยูนิต/กรัม ซูริมิ) ส่งผลให้ค่าแรงเจาะทะลุและระยะทางก่อนการเจาะทะลุมีค่าเพิ่มสูงขึ้น เจลซูริมิที่ผ่านการเติมเจลาตินจากหนังปลาวัวและเจลาตินจากปลาทางการค้าส่งผลให้ค่าปริมาณของเหลวบีบอัดและค่าความขาวลดลง (p<0.05) จากการศึกษาโดยเทคนิคอิเล็คโตรโฟรีซีส พบว่า แถบไมโอซินเส้นหนักของเจลซูริมิลดลงเมื่อเติมเจลาตินหรือเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ เมื่อศึกษาโครงสร้างจุลภาคพบว่าโครงข่ายของเจลซูริมิที่ผ่านการเติมเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์มีลักษณะละเอียดและแน่น ส่วนเจลซูริมิที่เติมเจลาตินมีลักษณะหยาบและไม่เป็นระเบียบ ปริมาณและชนิดของเจลาตินมีผลต่อสมบัติของเจลซูริมิ และเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ สามารถเพิ่มความแข็งแรงให้แก่เจลซูริมิที่ผ่านการเติมเจลาติน เจลาตินจากหนังปลาวัว สามารถใช้เป็นสารเติมแต่ง ที่ไม่ใช่โปรตีนกล้ามเนื้อ ร่วมกับเอนไซม์ทรานส์กลูทามิเนสจากจุลินทรีย์ ดังนั้นเจลซูริมิที่เติมเจลาตินซึ่งเป็นอนุพันธ์ของคอลลาเจนสามารถผลิตให้เป็นที่ยอมรับของผู้บริโภคได้

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ABSTRACT

Surimi gel from threadfin bream (*Nemipterus bleekeri*) added with commercial fish gelatin (FG) 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%) had varying properties. Breaking force and deformation of surimi gel decreased as gelatin levels increased ($p<0.05$), mainly due to dilution effect of myofibrillar proteins. When OTA was incorporated, the increases in breaking force and deformation were noticeable in surimi gel added with 0-10% FG and strengthening effect was in dose-dependent manner. On the other hand, the addition of OTA at higher levels resulted in the decreases in both breaking force and deformation of surimi gel added with 15-20% FG ($p<0.05$). Addition of FG generally lowered the expressible moisture content of surimi gel. Whiteness of surimi gel decreased when the levels of both FG and OTA increased ($p<0.05$). The addition of 0.1% OTA decreased myosin heavy chain (MHC) in surimi gel containing 5-20% FG slightly.

When microbial transglutaminase (MTGase) at various concentrations (0.0, 0.2, 0.4 and 1.2 units/g surimi) was added in combination with FG (0-20%), the increases in breaking force and deformation were obtained in surimi gel added with 0-15% FG, especially when MTGase level increased. Nevertheless, the addition of MTGase at higher levels led to the decrease in both breaking force and deformation of surimi gel containing 20% FG ($p<0.05$). The addition of MTGase at 1.2 units/g surimi decreased MHC in surimi gel containing 5-20% FG slightly, indicating its cross-linking ability.

The effect of MTGase at 1.2 units/g surimi on properties of surimi gel containing bovine gelatin (BG) and bovine/fish gelatin mix (BFGM; 1:1, 2:1, 1:2, 4:1

and 1:4) at 10% protein substitution was elucidated**.** When MTGase at 1.2 units/g surimi was incorporated, the increases in breaking force and deformation were noticeable in both surimi gel with and without 10% BG added (p<0.05). Moreover, surimi gels added with BFGM at all bovine/fish gelatin ratios had the higher breaking force and deformation, compared with that added with BG, when MTGase was incorporated

When gelatins from the skins of unicorn leatherjacket extracted at 50°C (UG) was added into surimi gel at 10% protein substitution in comparison with FG, breaking force and deformation of surimi gel decreased when the both gelatins were added (p<0.05). When MTGase was incorporated (1.2 units/g surimi), the increases in breaking force and deformation of surimi gel were obtained. Addition of UG or FG generally lowered the expressible moisture content and whiteness of surimi gel (p<0.05). Based on SDS-PAGE, band intensity of MHC of surimi gel decreased when surimi gel was added with gelatins or MTGase. The microstructure study revealed that surimi gel network became finer and denser with the addition of MTGase at 1.2 units/g surimi, but the coarser and irregular structure was obtained when gelatin was incorporated. Generally, level and type of gelatin had the impact on properties of surimi gel and MTGase was able to strengthen the surimi gel added with gelatin. Gelatin from unicorn leatherjacket skin could be used as non-muscle protein additive in conjunction with MTGase. As a consequence, surimi gel containing gelatin, a derivative of collagen, can be produced with consumer acceptability.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 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, which is 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. 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; Benjakul *et al.*, 2004b) or oxidized phenolic compound (Balange and Benjakul, 2009a; b; c; 2010). 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 bone and it is an important functional biopolymer that has a broad application 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 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 have been known to determine 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 (Hernández-Briones *et al.*, 2009). However, a little information regarding the use of fish gelatin 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 a 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 improved and skin of unicorn leatherjacket can be maximally utilized.

1.2 Review of literature

1.2.1 Surimi

Surimi is minced fish, which is extracted with water to remove strong flavoring compounds, pigments, and nonfunctional proteins and is 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 (1986), 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).

1.2.2 Gelation of surimi

Gel forming ability of 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: Addition of salt in combination of heating are two major factors involved in denaturation and gelation of muscle proteins. The addition of salt shifts the denaturation transitions to lower temperatures and decreases the enthalpies of heat denaturation. The addition of salt causes a partial unfolding of proteins and increases 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 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 to 50°C, indicating conformational changes in active sites in actomyosin.

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 (*Priacanthus tayenus*) surimi gel. TGase from different species determines the setting condition differently, depending upon 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. 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.

The addition of calcium salts to improve gelling properties of surimi may actually be due to their effects on TGase in the muscle, which requires Ca^{2+} for activation. Addition of calcium salt in the surimi resulted in the increased gel strength of surimi (Benjakul *et al.*, 2004a; Ramírez *et al.*, 2003). Furthermore, calcium ions can form salt linkages between negatively charged sites on two adjacent proteins (Wan *et al.*, 1994).

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 (Benjakul *et al.*, 2008; Chanarat *et al.*, 2012; Duangmal and Taluengphol, 2010; Jiang *et al.*, 2000; Seguro *et al.*, 2006; Yongsawatdigul and Piyadhammaviboon, 2005).

1.2.3 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 gelling property. Enzyme inhibitors, such as beef plasma protein, egg whites, whey protein concentrates, or potato extracts, have been used in conjunction with gel enhancers, and color enhancers. Protease inhibitor is formulated with sucrose, sorbitol, sodium tripolyphosphate, tetrasodium pyrophosphate, calcium compounds (calcium lactate, calcium sulfate, calcium citrate and calcium caseinate), sodium bicarbonate, monoglyceride or diglyceride and partially hydrogenated canola oil (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. These compounds should be added when the surimi paste is prepared to make gels (Lee and Park, 2006).

1.2.3.1 Use of microbial transglutaminase

Microbial transglutaminase (MTGase) has been found in some microorganisms. Ando *et al.* (1989) isolated microorganism (*Streptoverticillium mobaraense*) that produced a 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. 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-disulfide 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 *Streptoverticillium ladakanum* (Tsai *et al.*, 1996) have been shown the potential to increase the gel strength of fish surimi (Benjakul and Visessanguan, 2003).

Addition of MTGase 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 min or at 45°C for 20 mim for threadfin bream surimi. 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. Ramírez *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 obtained. 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 MTGase, temperature and time were optimized to improve the mechanical properties of surimi from silver carp (Ramírez *et al.*, 2000). Optimal predicted properties were obtained by employing the following setting conditions: a concentration of MTGase of 8.8 g/kg of surimi, at 39.6°C for 1 h. Under these conditions, gel from silver carp surimi with shear stress of 146 kPa and shear strain of 1.59 was obtained (Ramírez *et al.*, 2000). Shear stress was strongly affected by temperature and time, while shear strain was moderately affected (Ramírez *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. Breaking force and deformation of lizardfish surimi gel were significantly increased by MTGase. Moreover, the improved textural property was concomitant with cross-linked myosin heavy chain and tropomyosin, but not actin. The addition of MTGase in surimi improved the

storage modulus (*G´*) of lizardfish surimi (Yongsawatdigul and Piyadhammaviboon, 2005).

The effect of MTGase on breaking strength and deformation of gels from Alaska pollock surimi with and without setting at 30°C was studied and the formation of ε-(γ-glutamyl) lysine (GL) crosslink was monitored. 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 suggested 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 $CaCl_2$.

MTGase showed the gel strengthening effect on red tilapia (*O. niloticus*×*O. placidus*), 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 and Taluengphol, 2010). Chanarat *et al.* (2012) found that the addition of MTGase (0–0.6 units/g surimi) in surimi from threadfin bream (*Nemipterus furcosus*), Indian mackerel (*Rastrelliger kanagurta*) and sardine (*Sardinella gibbosa*) enhanced the gel-forming ability of surimi from three fish species. Surimi gels had the decreases in expressible moisture content when MTGase was added. Gels from threadfin bream which had the highest ε-amino group content showed the highest gel strength, when MTGase (0.2-0.6 U g^{-1}) was added, followed by Indian mackerel and sardine, respectively. The reactivity of muscle proteins toward MTGase induced cross-linking

and MHC proportion were the key factors determining the gel strength of surimi or fish muscle proteins (Chanarat *et al.*, 2012).

1.2.3.2 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 compound/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 (2009c) studied the effect of oxidized phenolic compounds on the gel properties of mackerel (*Rastrelliger kanagurta*) surimi. Breaking force and deformation of gels increased as the oxidized phenolic compounds increased up to a particular level. Gels with addition of 0.40% oxidized ferulic acid (OFA) or 0.50% oxidized 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% oxidized 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 oxidized 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 oxidized 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 (2009a) studied the effect of added oxidized 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, oxidized 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 (2009b) also reported that gels added with oxidized ferulic acid (OFA) or oxidized 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.

1.2.3.3 Use of non muscle proteins

1.2.3.3.1 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 egg 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, 1996). Campo-Deano and Tovar

(2009) studied the influence of egg white protein at different proportions (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. The quality of red tilapia surimi gel can be improved by the addition of egg white (EW) which can result in a significant increase in the breaking force compared to the control (no-additive). EW resulted in significantly less expressible water, compared to the control (no-additive). However, it resulted in the decrease in whiteness (Duangmal and Taluengphol, 2010).

1.2.3.3.2 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.

1.2.3.3.3 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.* (2005) studied the hardness of whiting surimi gels from Atlantic whiting 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, yielded more brittle gel. 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) decreased the whiteness of gels. Moreover, Benjakul *et al.* (2010) report that the addition of WPC up to 3% (w/w) resulted in the increased breaking force and deformation with higher water-holding capacity of surimi from goatfish. Addition of $CaCl_2$ at 50 mmoles/kg and 3% WPC effectively improved the gel strength of kamaboko gel but slightly lowered gel strength of modori gel from goatfish surimi. Thus, the use of WPC and $CaCl_2$ in goatfish surimi together with prior setting led to the improved gel-forming ability of goatfish surimi.

1.2.3.3.4 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.*, 2001). 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, Weerasinghe *et al.* (1996) 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 were found to inhibit the autolysis of surimi gel associated with 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.

1.2.3.3.5 Gelatin

Recently, fish gelatin has been used as an additive in surimi gel. Hernández-Briones *et al.* (2009) studied the effect of fish gelatin on the mechanical and functional properties of gels. Addition of fish gelatin up to 10 g/kg into grade A surimi from Alaska pollock had no effect on 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.

1.2.4 Fish gelatin and properties

1.2.4.1 Gelatin extraction

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 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).

Gelatin is partially hydrolyzed collagen or denatured form of collagen. During transition from collagen to gelatin, many noncovalent bonds are broken along with some covalent inter- and intramolecular bonds (Schiff base and aldol 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 non collagenous 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). Depending on the method in which the collagens are pretreated, two different types of gelatin (each with differing characteristics) can be produced (Figure 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 cross linked 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).

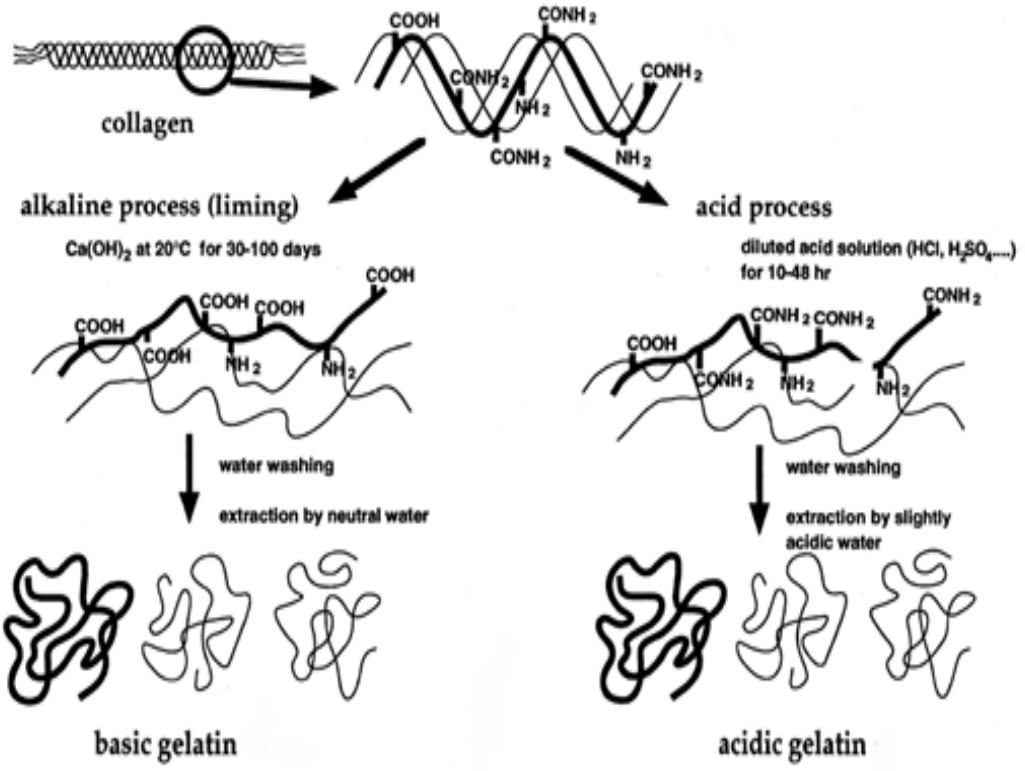

Figure 1. Preparative process for gelatins from collagen.
Source: Modified from Tabata and Ikada (1998)

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 (Johnston-Banks, 1990). 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).

Extraction temperature is another important factor affecting the functional properties, especially, gel strength (bloom) of gelatins (Schrieber and Gareis, 2007). Normand *et al.* (2000) reported that higher extraction temperature caused protein degradation, producing protein fragments and lowering gelling ability. Gómez-Guillén *et al.* (2002) found that squid gelatin extracted at 80°C showed very weak gel. For gelatin from Nile perch skin and bone, the gelatin extracted at 50°C exhibited the higher gel strength than corresponding bone gelatin. The gelatins extracted from Nile perch skins at higher temperature (60 and 70°C) also exhibited lower gel strength, melting point, setting temperature and longer setting time. Gelatins obtained from higher temperature extraction process were found to contain more peptides with molecular weight less than α chain and lower proportion of high molecular weight (greater than β) fractions than those with lower extraction temperature. (Muyonga *et al.*, 2004). Similarly, gelatin extracted from dorsal skin of yellowfin tuna (*Thunnus albacares*) had the higher gel strength when the lower extraction temperature was used (Cho *et al.*, 2005). Gel forming ability and the physical properties of gelatin from skate skin decreased as extraction temperature increased (Cho *et al.*, 2006). Yang *et al.* (2007) also reported that gelatin from channel catfish skin showed the lower gel strength as extraction temperature increased from 60 to 75°C. Kittiphattanabawon *et al.* (2010) reported that gelatins from the skins of brownbanded bamboo shark and blacktip shark skins showed the decreases in gel strength as the extraction temperature and time increased. The shark gelatin extracted at 75°C showed the highest degradation peptides, while gelatin extracted at 45°C had the highest content of α-chains. Moreover, the band intensity of β-, γ- and α-chains of gelatin from brownbanded bamboo shark and blacktip shark skins decreased with increasing extraction temperatures and times.

Moreover, heat-activated and heat-stable indigenous proteases associated with skin matrix can contribute to the destabilization as well as disintegration of collagen structure by disrupting the intra- and intermolecular cross-

links (Wu *et al.*, 2008). Collagenolytic enzymes have the unique ability to catalyze the hydrolysis of collagen and gelatin (Sovik and Rustard, 2006). They can cleave triple-helical collagen at a single site, resulting in the formation of fragments corresponding to 1/4 and 3/4 of its initial length (Sano *et al.*, 2004). Collagenases are classified into two major groups, metallocollagenases and serine collagenases (Aoki *et al.*, 2003). Heat-activated serine protease in bigeye snapper skin was involved in the drastic degradation of the β- and α-chains of the gelatin extracted at 60^{o}C (Intarasirisawat *et al.*, 2007). These enzymes are bound with matrix components such as collagens (Woessner, 1991). The proteolytic degradation of high molecular weight components caused by indigenous proteases during extraction of gelatin at high temperature showed the adverse effects on gel-forming properties of resulting gelatin (Intarasirisawat *et al.*, 2007).

The maximal autolytic activity of bigeye snapper skin was observed at 60^{o}C and pH 7.5 (Intarasirisawat *et al*. 2007). With the addition of 0.001 mM SBTI, the degradation was markedly inhibited. β- and α- chains in gelatin were more retained when extracted at temperatures lower than 50^{o}C. However, a lower yield was obtained. Additionally, Ahmad *et al*. (2011) reported that serine protease was the major enzyme in pretreated skin from unicorn leatherjacket and was involved in the drastic degradation of collagen/gelatin at high temperature, used for gelatin extraction. Maximised degradation was found at pH 7 and 50^{o}C. Degradation was markedly inhibited by 0.04 mM soybean trypsin inhibitor (SBTI), with coincidental maintainance of β- and α-chains. As a result, the gel strength and emulsifying activity were increased, however the extraction yield was lowered.

1.2.4.2 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 proteins typically ranges between 20,000 and 250,000 Da (Keenan, 1994). 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% (Hudson, 1994; 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 and Linden, 1991; US FDA. 1997), while ash content ranges from 0.1 to 3.25% (Cheow *et al.*, 2007; Jongjareonrak *et al.*, 2006; 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 fish gelatin from different sources in comparison with gelatin from porcine skin (Karim and Bhat, 2009)**.**

1.2.4.3 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 isoelectric 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 (Figure 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 from skin bovine 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.

1.2.4.4 Gelation mechanism and gel properties

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 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). It is generally recognized that the imino acids, proline and hydroxyproline, are important in gelation (Kittiphattanabawon *et al.*, 2005). Although the amino acid composition is important for determining the gelling properties of a given gelatin, the average molecular weight and more specifically, the distribution of α-, β- or γ-chains, also affect the physical properties of gelatin (Gómez-Guillén *et al.* 2002).

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)

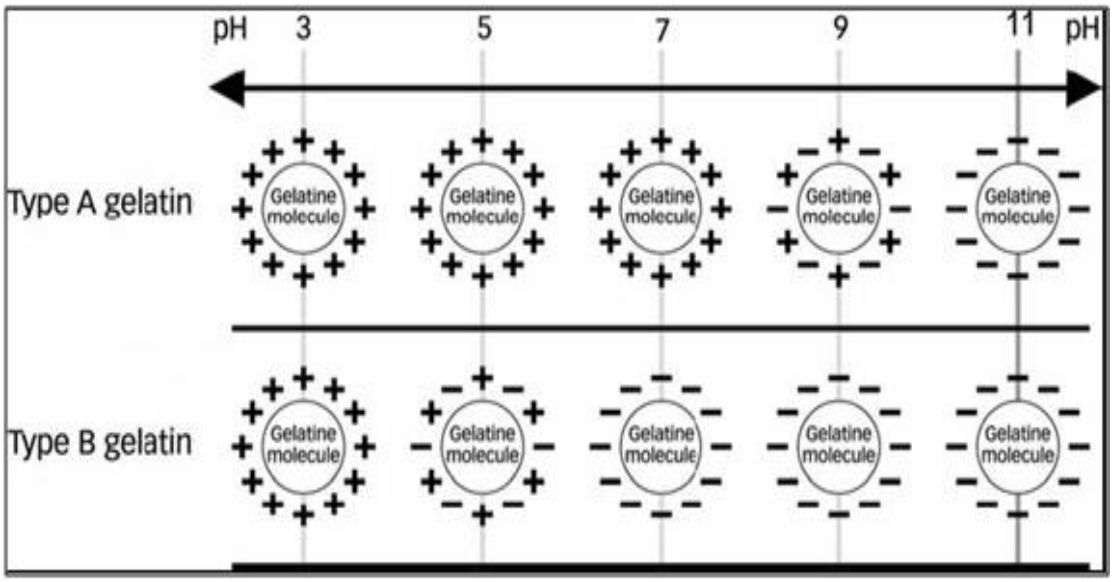

Figure 2. Charge distribution pattern of type A and B gelatins in aqueous solutions of different pH.

Source : Schrieber and Gareis (2007)

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. These are governed by molecular weight, as well as by complex interactions determined by the amino acid composition and the ratio of α/β-chains present in the gelatin (Cho *et al*., 2004). In addition, there is a strong correlation between gel strength and the α-chain content in gelatin. Gelatin containing more α-chains would thus show higher gel strength (Johnston-Bank, 1990). 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) (Gómez-Guillén *et al.*, 2002). This different behavior may be caused by the differences in the amino acid composition, the α1/α2 collagen-chain ratio and the molecular weight distribution. Cod gelatin had a lower alanine and imino acid content and a decreased proline hydroxylation degree. Cod and hake gelatins had a low α1/α2 ratio (~1) and

hake gelatin showed a highly significant decrease in β-components and other aggregates (Gómez-Guillén *et al.*, 2002).

Extraction condition has been known to have the impact on properties of usually fish gelatin. Kittiphattanabawon *et al.* (2010) reported that gelatin from the skins of brownbanded bamboo shark (BBS) and blacktip shark (BTS) 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-chain and γ-chain) 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. Fernández-Díaz *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.

1.2.4.5 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 counterpart (Norland, 1990). Fernández-Díaz *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. Among all salts assayed, only $MgSO_4$ improved the rheological characteristics under suitable conditions of pH and ionic strength, which differed between megrim and tilapia gelatin.

Plant phenolics can be used as gelatin cross-linker, thereby increasing gel strength of 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 pH 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.

Additionally, MTGase has been used to improve properties of gelatin. Jongjareonrak *et al.* (2006) found that the addition of 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 resulting 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 (Hernández-Balada *et al.*, 2009). Norziah *et al.* (2009) studied the effect of MTGase (0.5-5 mg/g gelatin) on *Tenualosa ilisha* fish gelatin in terms of melting, gelling temperature, gel strength and pH. MTGase 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 MTGase 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 when MTGase of 1.0 and 3.0 mg/g was added, respectively.

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1.4 Objectives

1. To study the properties of threadfin brem surimi gel as influenced by commercial fish gelatin and oxidized tannic acid.
2. To determine the properties of threadfin brem surimi gel as affected by commercial fish gelatin and microbial transglutaminase.
3. To investigate the effect of commercial bovine and fish gelatin in combination with microbial transglutaminase on gel properties of threadfin brem surimi.
4. To investigate the properties of unicorn leatherjacket (*Aluterus monoceros*) gelatins extracted at various temperatures and to study their use in combination with microbial transglutaminase on gel properties of threadfin bream surimi.

CHAPTER 2

PROPERTIES OF SURIMI GEL AS INFLUENCED BY FISH GELATIN AND OXIDIZED TANNIC ACID

2.1 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 resulted in 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.

2.2 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 desirable myofibrillar 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 (Hernández-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.

2.3 Materials and methods

2.3.1 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 (~80-300 g bloom, protein content >84%) was obtained from LAPI GELATINE S.p.A. (Empoli, Italy).

2.3.2 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-controlled water bath (40°C) (W350, Memmert, Schwabach, Germany) and subjected to oxygenation for 1 h by bubbling the solution with oxygen with the purity of 99.5–100% (TTS Gas Agency, Hat Yai, Songkhla, Thailand) to convert tannic acid to quinone. The solution was adjusted to pH 7 by using 6 N HCl and referred to as “oxidized tannic acid” (OTA).

2.3.3 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. Frozen surimi 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 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.

2.3.4 Determination of properties of surimi gel

2.3.4.1 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 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) 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.

2.3.4.2 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) (Bourne, 1978) and using a cylinder probe with a diameter of (2.5 cm). Hardness, springiness, cohesiveness, gumminess and chewiness were determined.

2.3.4.3 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:

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

2.3.4.4 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

2.3.4.5 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 (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 (MIK-RO20, Hettich Zentrifugan, Germany) at 3,500 xg for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hodgen, 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, 1ml of β-mercaptoethanol, 2.5 ml of 0.5 M 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.

2.3.4.6 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). Gel samples were cut into small pieces (0.25x0.25x0.25 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 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).

2.3.4.7 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.

2.3.5 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 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).

2.4. Results and discussion

2.4.1 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 Figure 3. 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 Hernández-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 (Hernández-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 from horse 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).

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, used the bundle like large aggregates could be formed. The coarser structure was generally associated with the poorer gel property (Balange and Benjakul, 2009b).

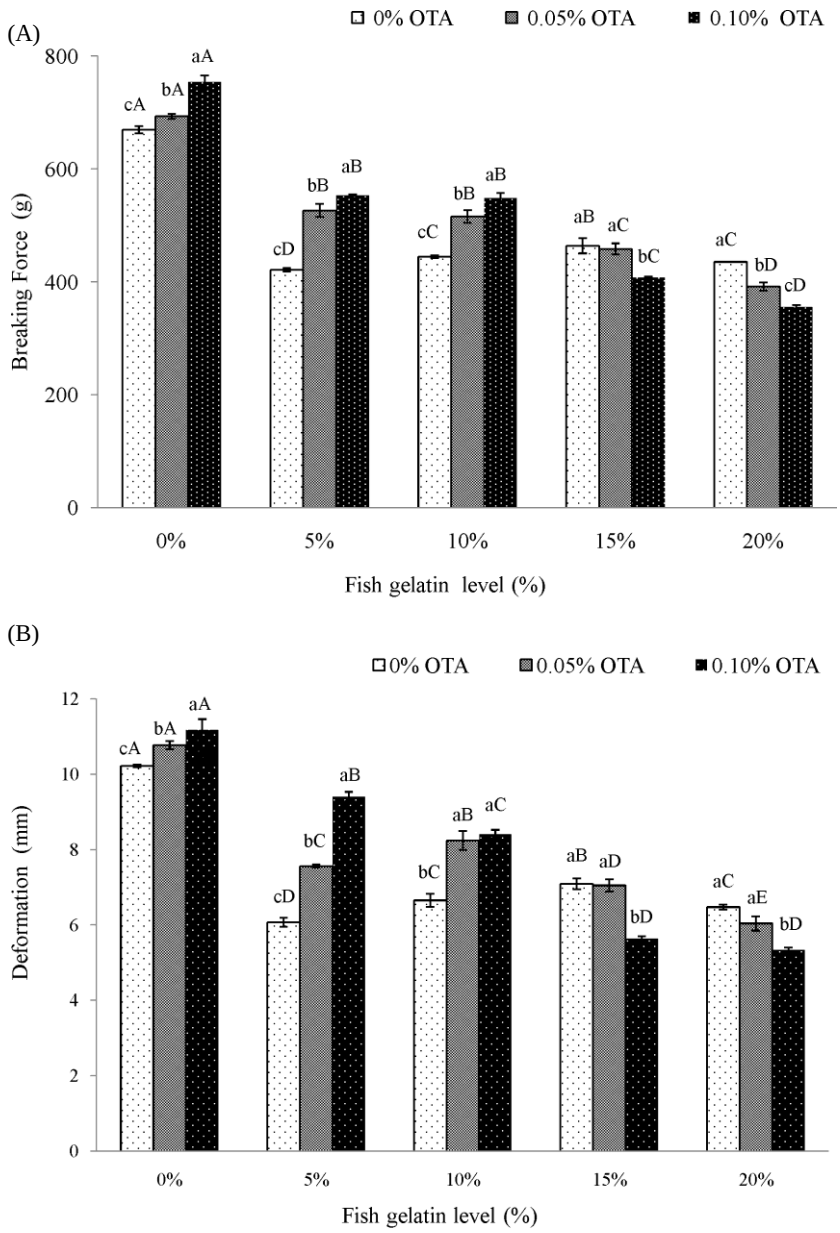

Figure 3. Breaking force (A) and deformation (B) of surimi gel added with fish gelatin and OTA at different levels. Bars represent the standard deviations (n=3). Different lowercase letters on the bars within the same level of gelatin denote the significant differences (p<0.05). Different uppercase letters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

2.4.2 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 2). 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 containing 15 and 20% fish gelatin ($p>0.05$). The result indicated that water-holding capacity of 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 Hernández-Briones *et al.* (2009) who reported that Alaska pollock surimi gels containing 7.5-15 g/kg 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).

2.4.3 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 2). 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.

2.4.4 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% OTA are depicted in Figure 4. 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 (Figure 3). The interference of myofibrillar proteins interaction was likely the major cause of the inferiority to gel strength.

Table 2. 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^{cC}	74.79 ± 0.44^{cA}
10	0	1.86 ± 0.18^{aB}	80.49 ± 0.09^{aB}
	0.05	1.82 ± 0.25^{aB}	77.24 ± 0.10^{bA}
	0.1	1.67 ± 0.12^{bC}	73.81 ± 0.27^{cB}
15	0	1.57 ± 0.14^{aC}	77.39 ± 0.17^{aC}
	0.05	1.58 ± 0.16^{aD}	74.43 ± 0.28^{bB}
	0.1	1.62 ± 0.06^{aC}	72.57 ± 0.11^{cC}
20	0	1.57 ± 0.07^{bC}	75.09 ± 0.27^{aD}
	0.05	1.69 ± 0.19^{aC}	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 ($p<0.05$).

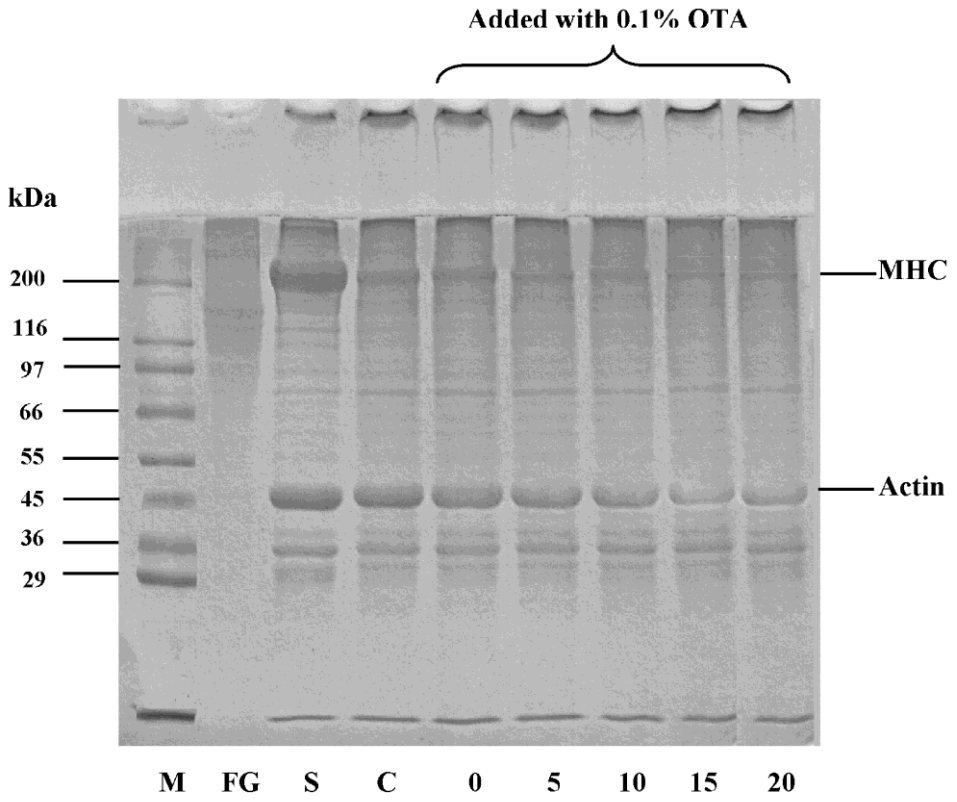

Figure 4. SDS-PAGE pattern of proteins of surimi gel added with fish gelatin at different levels without and with 0.1% OTA; M: marker; FG: fish gelatin; S: surimi paste; C: control (without fish gelatin and OTA); Numbers designate the amount of fish gelatin expressed as % protein substitution of surimi.

2.4.5 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 3. 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.

2.4.6 Likeness score of surimi gel added with fish gelatin at different levels with and without 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 4. 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 3). 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. 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^{c}	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^{c}	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=5). Different lowercase superscripts in the same column denote the significant difference (p<0.05)

Table 4. 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).

2.4.7 Microstructures of surimi gel added with fish gelatin at different levels with and without 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 Figure 5. 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 (Figure 3) as well as the lower expressible moisture content (Table 2). 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.

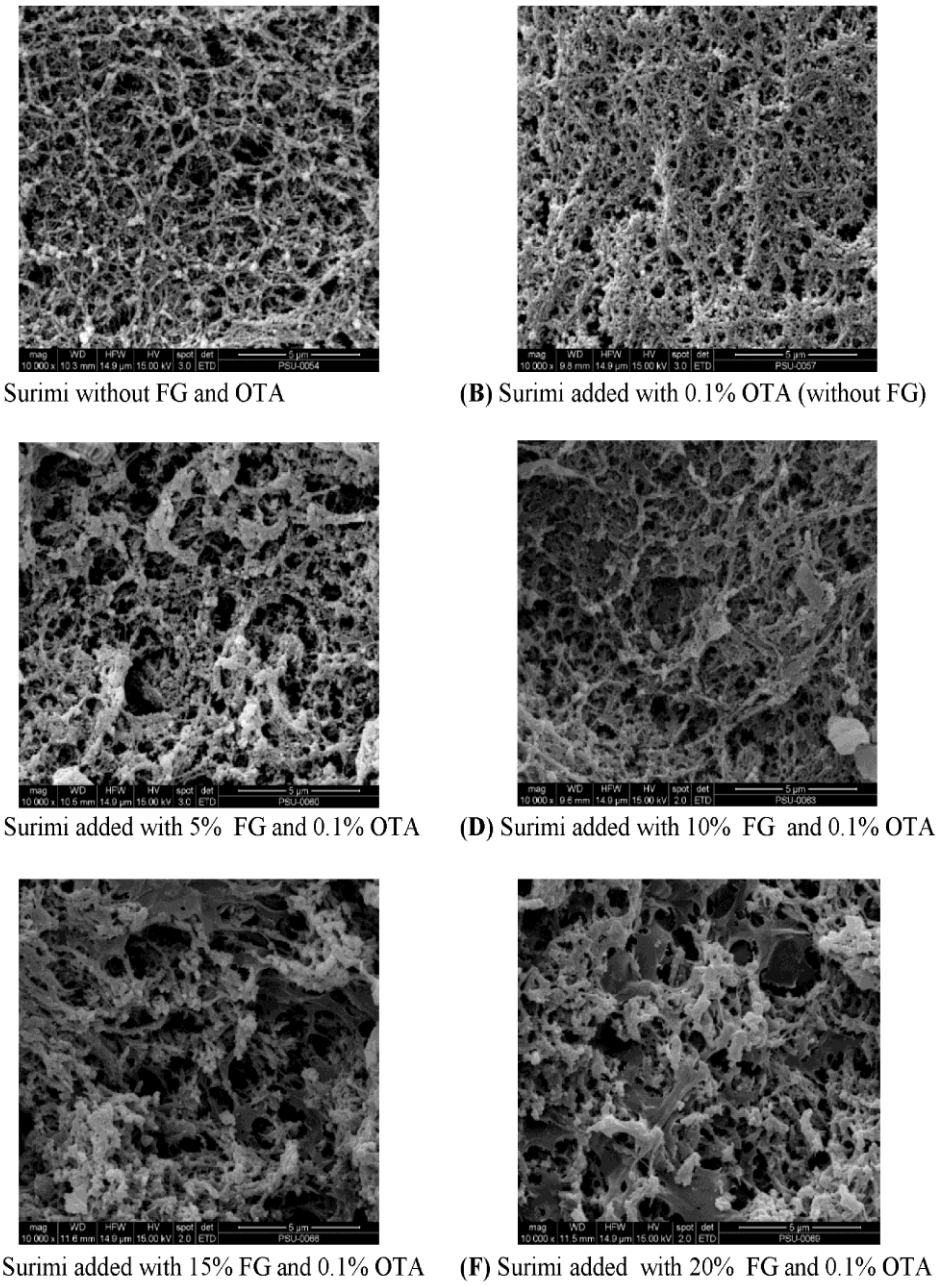

Figure 5. Electron microscopic images of surimi gel added with fish gelatin (FG) at different levels and 0.1% OTA (Magnification: 10,000 X).; A: Surimi gel without FG and OTA; B: Surimi gel added with 0.1% OTA (without FG); C: Surimi gel added with 5% FG and 0.1% OTA; D: Surimi gel added with 10% FG 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.

2.5 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 degrees.

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CHAPTER 3

PROPERTIES OF SURIMI GEL AS INFLUENCED BY FISH GELATIN AND MICROBIAL TRANSGLUTAMINASE

3.1 Abstract

Gel properties of threadfin bream (*Nemipterus bleekeri*) surimi added with fish gelatin at different levels (0, 5, 10, 15 and 20% protein substitution) in combination with microbial transglutaminase (MTGase) at various concentrations (0.0, 0.2, 0.4 and 1.2 units/g surimi) were studied. Breaking force and deformation of surimi gel decreased when the levels of fish gelatin added 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, especially when MTGase level increased. 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. In general, whiteness of surimi gel was lowered when the levels of both fish gelatin and MTGase increased ($p<0.05$). Electrophoretic study revealed that band intensity of myosin heavy chain (MHC) and actin of surimi gel decreased when fish gelatin levels increased, mainly due to dilution effect. The addition of MTGase at 1.2 units/g surimi decreased MHC in surimi gel containing 5-20% fish gelatin slightly, indicating its cross-linking ability. Therefore, both gelatin and MTGase affected the properties of surimi and the optimal levels should be used to obtain the acceptable gels.

3.2 Introduction

Surimi is minced fish meat subjected to washing 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. It has been used to prepare a variety of processed foods such as kamaboko, kani(crab)-kamaboko, chikuwa, satsumage, 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 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.*, 2001; Benjakul and Visessanguan, 2003; Benjakul *et al.*, 2004b; Benjakul *et al.*, 2007; Benjakul *et al.*, 2008; Campo-Deano and Tovar, 2009; Duangmal and Taluengphol, 2010; Rawdkuen and Benjakul, 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 gel 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-glutaminyl) lysine intra and intermolercular cross-links of proteins (DeJong and Koppelman, 2002). 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 weakening problem 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.

3.3 Materials and methods

3.3.1 Chemicals/gelatin

Sodium dodecyl sulfate (SDS), β-mercaptoethanol (βME), glycerol, high molecular weight marker 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 (~80-300 g bloom, protein content >84%) was obtained from LAPI GELATINE S.p.A. (Empoli, Italy). Microbial transglutaminase (MTGase) from *Streptoverticillium mobaraense* (TG-K) with activity of 100 units/g dry matter was supplied by Ajinomoto (Thailand) Co., Ltd. (Bangkok, Thailand). The enzyme powder consisted of 99% maltodextrin and 1% enzyme on a mass basis.

3.3.2 Preparation of surimi gel added with fish gelatin and MTGase

Frozen surimi from threadfin bream (*Nemipterus bleekeri*) was purchased from Pacific Fish Processing Co., Ltd., Songkhla, Thailand. Frozen surimi 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 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 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.

3.3.2 Determination of properties of surimi gel

3.3.2.1 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) 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.

3.3.2.2 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) (Bourne, 1978) and using a cylinder probe with a diameter of (2.5 cm). Hardness, springiness, cohesiveness, gumminess and chewiness were determined.

3.3.2.3 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 ((X-Y)/X).

3.3.2.4 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

3.3.2.5 SDS-polyacrylamide gel electrophoresis

Protein patterns of surimi paste and different 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 (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 3,500 xg for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hodgen, 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 0.5 M 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.

3.3.2.6 Microstructure of surimi gel

Microstructure of surimi gel samples containing 0, 5, 10, 15 and 20% fish gelatin without and with the addition of MTGase at 1.2 units/g surimi was determined using a scanning electron microscope (SEM). Gel samples were cut into small pieces (0.25x0.25x0.25 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).

3.3.2.7 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.

3.3.3 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.).

3.4 Results and discussion

3.4.1 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 surimi) are shown in Figure 6. Without MTGase, breaking force and deformation of surimi gel decreased as gelatin levels increased ($p<0.05$). Among all samples added with fish gelatin, those containing 15% fish gelatin showed the highest breaking force and deformation ($p<0.05$), while that added with 5% gelatin had the lowest breaking force and deformation ($p<0.05$). Gelatin is hydrophilic in nature, in which water could be imbibed effectively in gel matrix. Gelatin at 15% could interact with myofibrillar proteins, more lately via H-bond. Furthermore, it could imbibe a higher content of water 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 donor 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 the levels of fish gelatin 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 amount of myofibrillar proteins in the samples. Several 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). The determintal effect on mechanical properties of surimi gels might be associated with preventive effect of gelatin toward the formation of three-dimensional structure by 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*), 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 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 and Taluengphol, 2010). The addition of MTGase is reported to induce the cross-linking of MHC, thereby increasing the gel strength (Hsieh *et al.*, 2002; Jiang *et al.*, 2000; Sakamoto *et al.*, 1995).

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%. Several studies have shown that the use of MTGase above an optimum concentration causes a detrimental effect on the resulting textural properties of surimi gel (Lee *et al.*, 1997; Ramírez *et al.*, 2002). Sakamoto *et al.* (1995) suggested that excessive formation of ε-(γ-glutamyl) lysine crosslinks 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 proteins were preferably polymerized (data not shown). This caused the disconnected gel network, leading to the weakening of resulting gel.

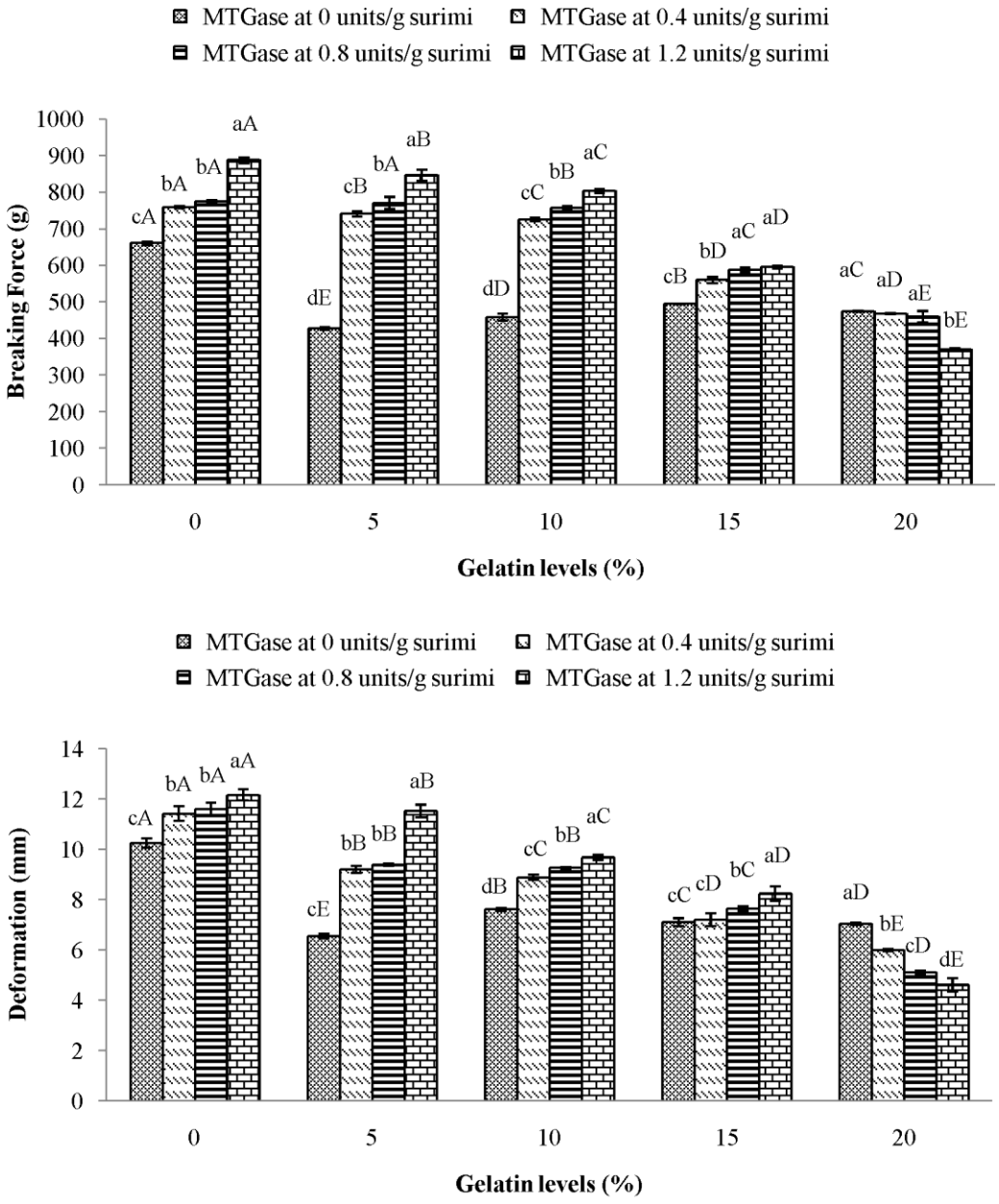


Figure 6. Breaking force (A) and deformation (B) of surimi gel added with fish gelatin and MTGase at different levels. Bars represent the standard deviations (n=3). Different lowercase letters on the bars within the same level of gelatin denote the significant differences (p<0.05). Different uppercase letters on the bar within the same level of MTGase denote the significant differences (p<0.05).

3.4.2 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 5. 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 Hernández-Briones *et al.* (2009) who reported that the surimi gels from Alaska pollock surimi containing 7.5-15 g/kg of fish gelatin showed the improved water holding capacity. When MTGase was incorporated in surimi, the decrease 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.* (2011) found that the addition of MTGase (0–0.6 units/g surimi) in surimi from threadfin bream (*Nemipterus furcosus*), Indian mackerel (*Rastrelliger kanagurta*) and sardine (*Sardinella gibbosa*) resulted in the decreases in expressible moisture content of resulting gels. Nevertheless, the increase in expressible moisture content was found in gel added with 20% fish gelatin when MTGase with increasing concentrations was incorporated ($p<0.05$). The increase in expressible moisture content was in accordance with the lowered breaking force and deformation (Figure 6). 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 weaken gel with poorer water holding capacity was obtained.

3.4.3 Whiteness of surimi gel added with fish gelatin at different levels with and without MTGase

Slight decreases in whiteness were found in surimi gels when fish gelatin levels increased, regardless of MTGase levels added ($p<0.05$) (Table 5). 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 surimi added with egg white, beef plasma protein and sodium ascorbate in combination with MTGase had the decrease in whiteness (Duangmal and Taluengphol, 2010). Furthermore, Rawdkuen *et al.* (2004) reported that gel of surimi from lizardish 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 level of 1.2 units/g surimi showed the highest efficiency in gel strengthening of surimi added with gelatin (0-15%). Therefore, MTGase 1.2 units/g surimi was used for further study.

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

Protein pattern of surimi gels without and with the addition of fish gelatin at different levels in the presence of MTGase 1.2 units/g surimi are depicted in Figure 7. Surimi paste contained 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 disappearance of MHC suggested the formation of ε-(γ-glutaminyl) lysine intra and intermolecular 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 mercaptoethanol used for electrophoresis (DeJong and Koppelman, 2002; Jiang *et al.*, 1998). The addition of MTGase (1.2 units/g surimi) decreased MHC band intensity in surimi gel to higher extent, compared with that found in the control gel. This suggested more protein cross-linking ability via ε-(γ-glutaminyl) lysine bonds by MTGase. The improvement of gel strength in some tropical fish was reported to be achieved by setting at 40°C, and 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°C for 90 min or 45°C for 20 min for threadfin-bream, and 30°C for 60 min for

pollack 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 intensity. Yongsawatdigul and Piyadhammaviboon (2005) reported that protein cross-linking in lizardfish surimi was catalyzed by MTGase at 40°C. It was noted that MHC and actin band intensity 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 not determined only by cross-linking but also by the alignment of proteins in the matrix.

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

TPA paremeters of surimi gel added with fish gelatin at various levels in the presence of MTGase 1.2 units/g surimi are shown in Table 6. When MTGase (1.2 units/g 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 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 negative 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 found in surimi gel without gelatin, added with MTGase 1.2 units/g surimi.

Table 5. Expressible moisture content and whiteness of surimi gel added with fish gelatin and MTGase at different levels

Fish gelatin (%)	MTGase (units/g surimi)	Expressible moisture content (%)	Whiteness
0	0	2.61 ± 0.28^{aA}	81.11 ± 0.07^{aA}
	0.4	2.48 ± 0.24^{bA}	81.11 ± 0.10^{aA}
	0.8	2.36 ± 0.15^{cA}	81.14 ± 0.07^{aA}
	1.2	2.14 ± 0.30^{dA}	81.12 ± 0.24^{aA}
5	0	1.99 ± 0.30^{aB}	80.56 ± 0.04^{aB}
	0.4	1.80 ± 0.15^{bB}	80.54 ± 0.13^{aB}
	0.8	1.77 ± 0.11^{cB}	80.52 ± 0.16^{aB}
	1.2	1.71 ± 0.13^{dC}	80.52 ± 0.14^{aB}
10	0	1.96 ± 0.02^{aB}	80.60 ± 0.07^{aB}
	0.4	1.80 ± 0.11^{bB}	80.51 ± 0.07^{aB}
	0.8	1.80 ± 0.26^{bB}	80.54 ± 0.24^{aB}
	1.2	1.70 ± 0.21^{cC}	80.53 ± 0.07^{aB}
15	0	1.79 ± 0.16^{aC}	79.22 ± 0.03^{aC}
	0.4	1.73 ± 0.18^{bC}	79.16 ± 0.01^{aC}
	0.8	1.73 ± 0.14^{bB}	79.08 ± 0.25^{aC}
	1.2	1.57 ± 0.19^{cB}	79.15 ± 0.20^{aC}
20	0	1.54 ± 0.24^{bD}	78.58 ± 0.09^{aD}
	0.4	1.75 ± 0.14^{aC}	78.06 ± 0.04^{aD}
	0.8	1.75 ± 0.15^{aB}	78.05 ± 0.16^{aD}
	1.2	1.76 ± 0.20^{aB}	78.09 ± 0.29^{aD}

Values are mean ± SD (n=3). 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 ($p<0.05$).

3.4.6 Likeness score of surimi gel added with fish gelatin at different levels withand without MTGase

Likeness score of surimi gels added without and with fish gelatin at various levels and MTGase 1.2 units/g surimi is shown in Table 7. Scores of texture and overall likeness of MTGase added gel were lower than those of control ($p<0.05$). The addition of MTGase resulted in the rigid gel caused by the enhanced protein

cross-linking. This resulted in the loss in elasticity of gel. In the presence of MTGase (1.2 units/g 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 of surimi gel property as well as sensory property. 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.

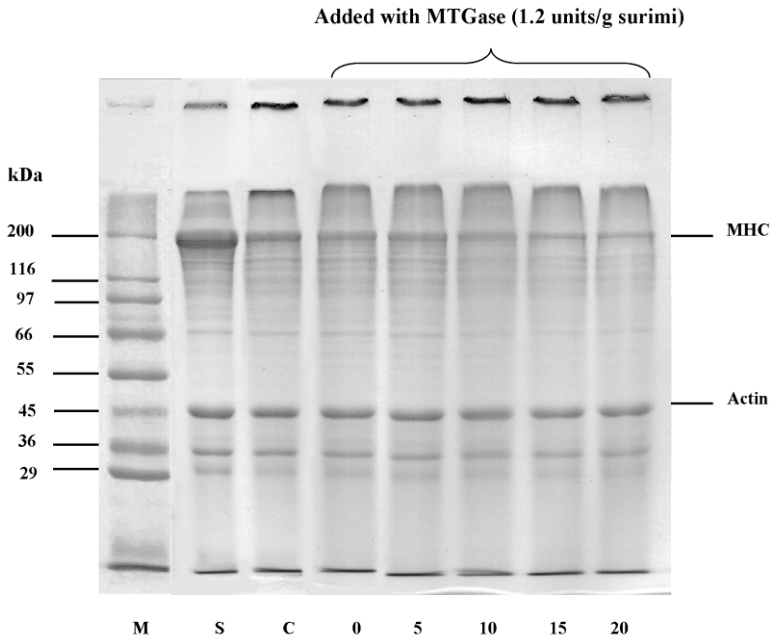


Figure 7. SDS-PAGE protein patterns of surimi gel added with fish gelatin at different levels without and with MTGase (1.2 units/g surimi); M: marker; S: surimi paste; C: control (without fish gelatin and MTGase (1.2 units/g surimi)); Numbers designate the amount of fish gelatin (% protein substitution of surimi.

Table 6. Textural properties of surimi gel added with various levels of fish gelatin and MTGase at a level of 1.2 units/g surimi

Samples	Hardness (N)	Springiness (cm)	Cohesiveness	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+MTGase (1.2units/g surimi) (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+MTGase (1.2units/g surimi)	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+MTGase (1.2units/g surimi)	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+MTGase (1.2units/g surimi)	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+MTGase (1.2units/g surimi)	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).

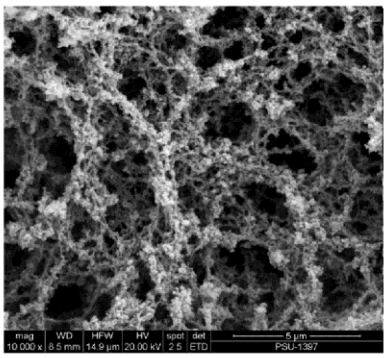
Table 7. Likeness score of surimi gel added with various levels of fish gelatin and MTGase at a level of 1.2 units/g surimi

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+MTGase (1.2units/g surimi) (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+MTGase (1.2units/g surimi)	7.63±1.33^{a}	6.37±1.66^{b}	6.86±1.35^{ab}	6.67±1.00^{b}
Surimi+10% FG+MTGase (1.2units/g surimi)	7.62±1.46^{a}	6.20±1.64^{b}	6.56±1.23^{abc}	6.50±1.37^{b}
Surimi+15% FG+MTGase (1.2units/g surimi)	7.76±0.99^{a}	4.30±1.47^{d}	6.43±1.22^{bc}	4.80±1.20^{d}
Surimi+20% FG+MTGase (1.2units/g surimi)	7.73±1.16^{a}	4.17±1.03^{d}	5.83±1.19^{c}	4.76±1.11^{d}

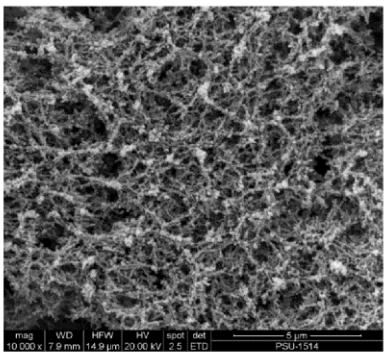
Values are mean ± SD (n=3). Different lowercase superscripts in the same column denote the significant difference (p<0.05)

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

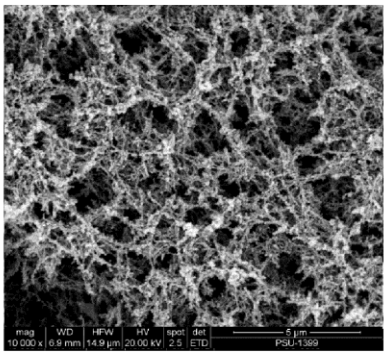
Microstructures of surimi gels added with fish gelatin at various levels in the presence of MTGase 1.2 units/g surimi are illustrated in Figure 8. Surimi gel network became finer and denser with the addition of MTGase, as compared with the control gel (without MTGase). Those myofibrillar proteins could undergo the cross-linking more effectively in the presence of MTGase, which induced the protein cross-linking. As a result, more compact and dense gel network was obtained. The finer and more ordered structure of MTGase added gel correlated with higher breaking force and deformation (Figure 6) as well as the lowered expressible moisture content (Table 5). 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 the poorer gel properties of surimi gel added with a higher amount of fish gelatin. Network with larger voids was not strong and not resistant to the force applied. Additionally, it could not hold water effectively. This confirmed the negative impact of fish gelatin on gelation of surimi.



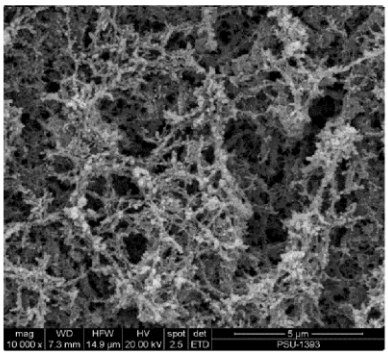
(A) Surimi without FG and MTGase



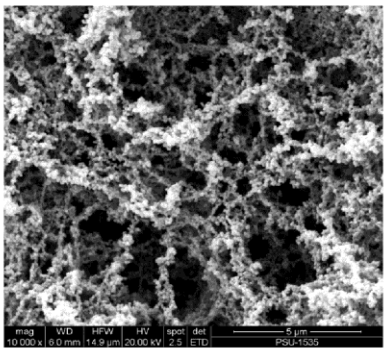
(B) Surimi added with MTGase 1.2 units/g surimi (without FG)



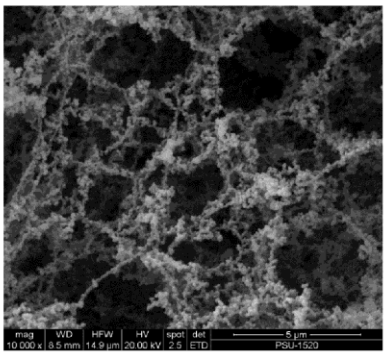
(C) Surimi added with 5% FG and MTGase (1.2 units/g surimi)



(D) Surimi added with 10% FG and MTGase (1.2 units/g surimi)



(E) Surimi added with 15% FG and MTGase (1.2 units/g surimi)



(F) Surimi added with 20% FG and MTGase (1.2 units/g surimi)

Figure 8. Electron microscopic images of surimi gel added with fish gelatin (FG) at different levels and MTGase at a level of 1.2 units/g surimi (Magnification: 10,000X).; A: Surimi gel without gelatin and MTGase; B: Surimi gel added with MTGase 1.2 units/g surimi (without gelatin); C: Surimi gel added with 5% gelatin and MTGase (1.2 units/g surimi); D: Surimi gel added with 10% gelatin and MTGase (1.2 units/g surimi); E: Surimi gel added with 15% gelatin and MTGase (1.2 units/g surimi); F: Surimi gel added with 20% gelatin and MTGase (1.2 units/g surimi).

3.5 Conclusion

The addition of fish gelatin up to 10% in conjunction with MTGase 1.2 units/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, surimi gel containing gelatin had the decrease in sensory property. Future study is still needed to combat this obstacle.

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CHAPTER 4

EFFECT OF BOVINE AND FISH GELATIN IN COMBINATION WITH MICROBIAL TRANSGLUTAMINASE ON GEL PROPERTIES OF THREADFIN BREAM SURIMI.

4.1 Abstract

Surimi threadfin bream (*Nemipterus bleekeri*) added with bovine gelatin (BG) and bovine/fish gelatin mix (BFGM; 1:1, 2:1, 1:2, 4:1 and 1:4) at 10% protein substitution in combination with and without MTGase at 1.2 units/g surimi. Textural properties, whiteness, expressible moisture content, protein pattern, microstructure and sensory property of gels were determined. When MTGase at 1.2 units/g surimi was incorporated, the increases in breaking force and deformation were noticeable in both surimi gel with and without 10% BG added ($p<0.05$). Moreover, surimi gels added with BFGM at all bovine/fish gelatin ratios had the higher breaking force and deformation, compared with that added with BG, when MTGase was incorporated. Addition of BG or BFGM generally lowered the expressible moisture content and whiteness of surimi gel ($p<0.05$). Based on SDS-PAGE, band intensity of MHC and actin of surimi gel decreased when surimi gel was added with all gelatins, regardless of MTGase addition. The microstructure study revealed that surimi gel network became finer and denser with the addition of MTGase 1.2 units/g surimi, but the coarser and irregular structure was obtained when gelatin was incorporated.

4.2 Introduction

Gelatin is one of the most versatile gelling agents in food applications due to its special texture and the "melt-in-mouth" perception. In addition, gelatin has a variety of applications in the pharmaceutical and photographic industry (Haug *et al.*, 2004). Gelatin has been used as an additive for improving elasticity, consistency and stability of foods (Arvanitoyannis, 2002). Fish gelatin was added into surimi gel, but it resulted in the decrease in gel strength (Hernández-Briones *et al.*, 2009). Gelatin is obtained by hydrolyzing the collagen present in the bones and skin, which are generated as byproducts during animal slaughtering and processing. Bovine and porcine skin and bone are the important sources for gelatin production. Recently, fish bones and skins have gained increasing attention as alternative raw material. Those byproduct generated by fish filleting can account for as much as 75% of the total weight of catches (Shahidi and Botta, 1994). Gelatin from different sources has varying properties, mainly related not only to the amino acid composition but also to the α-chain, β- or γ-component and molecular-weight distribution (Johnston-Banks, 1990). The intrinsic differences between mammalian and fish gelatins employed may determine different properties of gel (Benjakul *et al.*, 2009).

Overall surimi products in the Southeast Asian Region in 2005 are estimated to be 315,800 metric tons. Thailand was the largest surimi producer, followed by Malaysia and Vietnam with the amount of surimi products of 145,000, 95,300 and 64,000 MT, respectively (Pangsorn *et al.*, 2007). Surimi can be used to prepare a variety of processed foods such as kamaboko, kani(crab)-kamaboko, chikuwa, satsumage, fish sausages, fish balls, etc. Surimi with high quality yields the flexible gel with white color. To improve the properties of surimi gel, a number of additives have been used. Protein additives with proteolytic inhibitory activity and protein cross-linkers such as MTGase have been widely used in surimi (Benjakul and Visessanguan, 2003; Benjakul *et al.*, 2004b). MTGase from *Streptoverticillium mobaraense* does not require calcium ions for activity (Ando *et al.*, 1989). MTGase is an enzyme that catalyzes the cross-linking of proteins through the formation of covalent bonds between protein molecules. MTGase has been shown to be useful in strengthening surimi gels during the setting (Benjakul *et al.*, 2003; Seguro *et al.*,

2006). Addition of MTGase to surimi significantly increased gel strength, particularly when the surimi has lower natural setting ability (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 MTGase concentration (Tsukamasa *et al.*, 1990).

However, a little information regarding the use of gelatin, especially bovine and fish gelatin mix as the texture modifier in surimi gel has been reported. The addition of gelatin along with MTGase could be a means to modify the texture of surimi gel, which can fit the demand of consumers. Thus, this study aimed to investigate textural, physical and sensory properties of surimi from threadfin bream surimi added with bovine and fish gelatin mix in combination with MTGase**.**

4.3 Materials and methods

4.3.1 Chemicals/gelatin

Sodium dodecyl sulfate (SDS), β-mercaptoethanol (βME), glycerol, high molecular weight marker 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). Bovine hide gelatin (~240 g bloom) and fish gelatin (~80-300 g bloom, protein content >84%) were purchased from Halamic company (Bangkok, Thailand) and LAPI GELATINE S.p.A. (Empoli, Italy), respectively**.** MTGase from *Streptoverticilium mobaraense* (TG-K) was supplied by Ajinomoto (Thailand) Co., Ltd. (Bangkok, Thailand). Enzyme activity reported by the supplier was 100 units/g dry materials. The enzyme powder consisted of 99% maltodextrin and 1% enzyme on a mass basis.

4.3.2 Preparation of surimi gel added with fish gelatin and MTGase

Frozen surimi from threadfin bream (*Nemipterus bleekeri*) was purchased from Pacific Fish Processing Co., Ltd., Songkhla, Thailand. Frozen surimi was partially thawed at 4°C for 8-10 h to obtain the core temperature of approximately 0-2°C. Surimi was then cut into small pieces and mixed with 2.5%

NaCl in a mixer (MK-5087M, Panasonic Manufacturing Malaysia Berhad, Selangor, Malaysia). During chopping, the temperature was maintained below 10°C. Bovine gelatin (BG) was then added into surimi paste at 10% protein substitution. Bovine/fish gelatin mix (BFGM) with different BG and FG ratios (1:1, 2:1, 1:2, 4:1 and 1:4) were also used at a level of 10%. The mixtures were chopped for 5 min. To the mixture, MTGase was then added to obtain the level of 1.2 unit/g surimi 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.

4.3.3 Determination of properties of surimi gel

4.3.3.1 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) 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.

4.3.3.2 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) (Bourne, 1978) using a cylinder probe with a diameter of 2.5 cm. Hardness springiness, cohesiveness, gumminess and chewiness were determined.

4.3.3.3 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 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:

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

4.3.3.4 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 following equation :

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

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

4.3.3.5 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 (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 (MIK-RO20, Hettich Zentrifugan, Germany) at 3,500 xg for 20 min to remove undissolved debris. Protein concentration of the supernatant was determined by the Biuret method (Robinson and Hodgen, 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 0.5 M 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.

4.3.3.6 Microstructure of surimi gel

Microstructure of surimi gel samples added without and with gelatin in the presence and absence of MTGase (1.2 units/g surimi) was determined using scanning electron microscope (SEM). Gel samples were cut into small pieces (0.25x0.25x0.25 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).

4.3.3.7 Sensory evaluation

Surimi gels containing gelatin without and with gelatin addition in the presence and absence of MTGase (1.2 units/g surimi) were determined for likeness using hedonic (9-point-scale) (Meilgaard *et al.*, 1999). Thirty panelists, who were the graduate students in Food Science and Technology program and were familiar with surimi consumption, were asked to evaluate for color, texture, appearance and overall likeness.

4.3.4 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, U.S.A.).

4.4 Results and discussion

4.4.1 Breaking force and deformation of surimi gel added with gelatins in combination with and without MTGase

Breaking force and deformation of surimi gel from threadfin bream added with BG or BFGM in the presence or absence of MTGase are shown in Figure 9. Without MTGase addition, breaking force and deformation of surimi gel decreased as 10% BG was added ($p<0.05$). The detrimental effect on mechanical properties of surimi gels might be associated with disruptive effect of gelatin on the formation of three-dimensional structure of myofibrillar proteins (Hernández-Briones *et al.*, 2009). FG showed more negative effect on surimi gel property than BG, as evidenced by the marked decreased in both breaking force and deformation (data not shown). Various proteins and carbohydrates such as whey protein concentrate, wheat gluten (Chen, 2000; Murphy *et al.*, 2005) alginates, xanthan and high methoxyl pectins have a negative effect on surimi and fish gels (Barrera *et al.*, 2002; Park, 2000). In addition, gelatin might show the dilution effect on myofibrillar proteins in surimi, which played a role in gel formation. 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 at a level of 15 g/kg and 7.5 g/kg, respectively was added.

When MTGase was incorporated in surimi (without gelatin), breaking force and deformation increased by 33.4 and 21.4% respectively, compared with those of the control. Therefore, MTGase was effective in increasing the gel strength of

surimi without gelatin incorporated. For surimi added with 10% BG or BFGM, the addition of MTGase increased breaking force and deformation of resulting gels by 33.4-49.7%, compared with that containing 10% BG. MTGase is an enzyme that catalyses the cross-linking of proteins through the formation of covalent bonds between protein molecules. Addition of MTGase induced the cross-linking of MHC and substantially increased the gel strength of surimi (Hsieh *et al.*, 2002; Jiang *et al.*, 2000; Sakamoto *et al.*, 1995). Surimi gel added with BFGM having varying BF/FG ratios showed the different increases in the breaking force and deformation when MTGase was incorporated ($p<0.05$). Surimi gels added with BFGM having higher FG/BG ratio showed the lower breaking force than those containing BFGM with lower FG/BG ratio. The result indicated that FG exhibited more interfering effect on gel property of surimi than BG. This was plausibly due to the lower gel forming ability of the former. Also, FG might be less cross-linked by MTGase, compared with BG. As a result, the poorer gel was attained in the former.

Gel strength is the major physical properties of gelatin gels. These are governed by chain length, amino acid composition and the ratio of α/β-chains present in the gelatin (Cho *et al.*, 2004). According to Schrieber and Gareis (2007), the gel strength is mainly dependent on the proportion of fractions having a molecular weight of approximately 100,000 g mol^{-1}. In addition, there is a strong correlation between gel strength and the α-chain content in gelatin. Gelatin containing more α-chains showed higher gel strength (Karim and Bhat, 2009). Mammalian gelatin contains large amounts of total imino acids (proline and hydroxyproline) (Balian and Bowes, 1977). The lower content of proline and hydroxyproline gives fish gelatin a low gel modulus and low gelling and melting temperatures (Haug *et al.*, 2004). Similar trend was found for deformation. However the higher deformation was obtained in gel added with 10% BG and MTGase, compared with the control gel (without gelatin and MTGase addition). Futhermore, the higher FG ratio in BFGM resulted in the higher decrease in deformation of surimi gel.

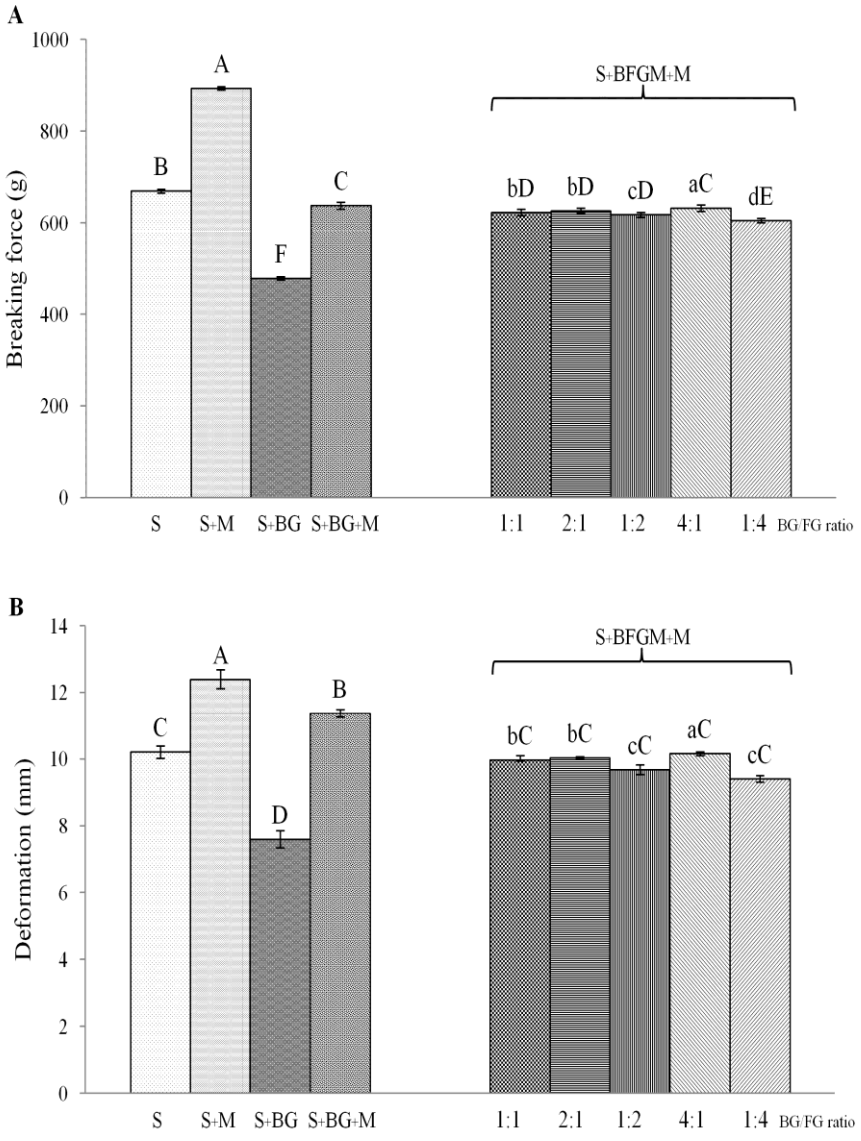


Figure 9. Breaking force (A) and deformation (B) of surimi gel added without and with gelatins in the absence and presence of MTGase. S: Surimi; M: MTGase (1.2 unit/g surimi); BG: Bovine gelatin; BFGM: Bovine/fish gelatin mix. Bars represent the standard deviations (n=3). Different lowercase letters or different uppercase letters on the bar denote the significant differences (p<0.05).

4.4.2 Expressible moisture content of surimi gel added with gelatins in combination with and without MTGase

Expressible moisture content of surimi gel added with BG and BFGM in the presence and absence of MTGase is shown in Table 8. The decreases in expressible moisture content were observed in surimi gels when 10% BG was added. The result indicated that fish gelatin was hydrophilic in nature and could bind water via H-bond. Gelatin could hold water molecularly in gel matrix and improved water-holding capacity of surimi gel. Hernández-Briones *et al.* (2009) reported that the surimi gels from Alaska pollock surimi containing 7.5-15 g/kg of fish gelatin showed the improved water holding capacity.

When MTGase was incorporated in surimi, the decreases in expressible moisture content were found in surimi gel, regardless of gelatin incorporated ($p<0.05$). Addition of MTGase induced the inter-connection of gel matrix, thereby enhancing water holding capacity of gel (Han *et al.*, 2009; Moreno *et al.*, 2008). The result was in accordance with Trespalacios and Pla (2007) who reported that adding 0.3% MTGase on chicken meat gel significantly decreased expressible moisture content. The addition of 0.5% MTGase to beef homogenates significantly decreased expressible moisture, cooking yield and purge loss from beef gels (Pietrasik, 2003). When both gelatin and MTGase were added in surimi gel, the water holding capacity was markedly increased. However, FG/BG ratio in BFGM had no effect on expressible moisture content of surimi gel. It was suggested that FG and BG exhibited similar water binding capacity.

4.4.3 Whiteness of surimi gel added with gelatins in combination with and without MTGase

The decreases in whiteness were found in surimi gels added with BG or all BFGM ($p<0.05$) (Table 8). The decrease in whiteness might be due to slightly yellowish color of gelatins. For gels added with all BFGM, there was no difference in whiteness among all samples tested ($p>0.05$). MTGase addition had no impact on whiteness of resulting surimi gels. Whiteness is another quality index of surimi gel. The additives have been reported to affect the whiteness of surimi gel, depending on

type and amount of additives incorporated (Benjakul and Visessanguan, 2000; Benjakul *et al.*, 2001; Benjakul *et al.*, 2007).

Table 8. Expressible moisture content and whiteness of surimi gel added 10% BG or BFGM without and with MTGase 1.2 units/g surimi

Samples	Expressible moisture content (%)*	Whiteness*
Surimi (without gelatin and MTGase)	2.62±0.16[a]	81.82±0.34 [a]
Surimi+ MTGase (without gelatin)	2.08±0.29[a]	81.85±0.16 [a]
Surimi+10% BG (without MTGase)	1.96±0.15[a]	80.80±0.14 [a]
Surimi+10% BG with MTGase	1.70±0.24[b]	80.89±0.56 [a]
Surimi+10% BFGM (the mixture of BG:FG, 1:1) with MTGase	1.75±0.02[b]	80.45±0.22 [b]
Surimi+10% BFGM (the mixture of BG:FG, 2:1) with MTGase	1.73±0.25[b]	80.42±0.37 [b]
Surimi+10% BFGM (the mixture of BG:FG, 1:2) with MTGase	1.75±0.14[b]	80.44±0.24 [b]
Surimi+10% BFGM (the mixture of BG:FG, 4:1) with MTGase	1.70±0.17[b]	80.34±0.14 [b]
Surimi+10% BFGM (the mixture of BG:FG, 1:4) with MTGase	1.76±0.20[b]	80.41±0.42 [b]

*Values are mean ± SD (n=3).

Different lowercase superscripts in the same column denote the significant differences (p<0.05).

BG: bovine gelatin; BFGM: bovine/fish gelatin mix; MTGase: microbial transglutaminase.

4.4.4 Protein patterns of surimi gel added with gelatins in combination with and without MTGase

Protein patterns of surimi gels added without and with gelatin in the presence or absence of MTGase are depicted in Figure 10. Decrease in MHC band intensity was found in surimi gel when MTGase was incorporated, compared with that observed in control gel (without fish gelatin and MTGase). The disappearance of MHC and actin in surimi gel added with MTGase suggested inter-molecular cross-linking of muscle proteins in surimi. Cross-links were not dissociated by the mixture of SDS and mercaptoethanol used for electrophoresis (DeJong and Koppelman, 2002;

Jiang *et al.*, 1998). The addition of MTGase is reported to cause the cross-linking of MHC (Hsieh *et al.*, 2002). The addition of 10% BG or BFGM 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 gelatins (10% BG/mix gelatin) was added, compared with that observed in control gel (without fish gelatin and MTGase). 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). In the present study, no difference in MHC band intensity of surimi gel added with BFGM having different ratio of BG and FG. MTGase could not effectively induce cross-linking of fish gelatin (data not shown). This was due to the constraint on amino acid composition for cross-linking induced by MTGase. Fish gelatin contained a low content of lysine (Schrieber and Gareis, 2007). Lysine is known to act as acyl acceptor for acyl transfer reaction induced by MTGase (Benjakul *et al.*, 2008)

4.4.5 Textural properties of surimi gel added with gelatins in combination with and without MTGase

TPA paremeters of surimi gels added without and with 10% BG or BFGM in the presence and absence of MTGase are depicted in Table 9. When 10% BG was added, hardness, springiness, cohesiveness, gumminess and chewiness decreased ($p<0.05$). The results indicated that gelatin might disturb the three-dimensional structure of myofibrillar protein networks. This was in agreement with the decrease in breaking force and deformation of surimi gel (Figure 9). When MTGase at a level of 1.2 units/g surimi was incorporated into surimi gel, all textural parameters increased ($p<0.05$). All textural parameters of surimi gel added with BG increased when MTGase was added ($p<0.05$). No differences in hardness, springiness and cohesiveness were observed between sample containing 10% BG in combination with MTGase and the control gel ($p>0.05$). This was due to the increased protein cross-linking induced by MTGase added. In the presence of MTGase, surimi gel added with BFGM having different BG/FG ratios had the decrease in hardness as the

FG/BG ratio increased. However, the highest chewiness was obtained when BFGM with FG/BG ratio of 1:4 was used (p>0.05). The result suggested that gelatin might disturb the three-dimensional structure of myofibrillar protein networks. However, the addition of MTGase was able to improve the textural properties of surimi gel containing gelatin or mixed gelatins to some extent.

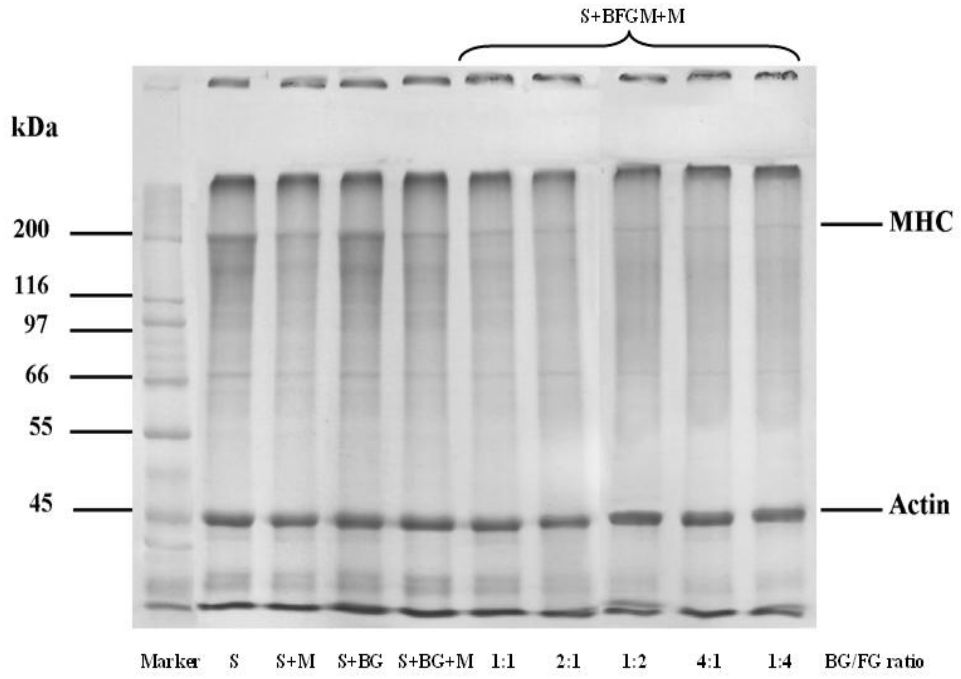


Figure 10. SDS-PAGE pattern of proteins of surimi gel added without and with gelatins in the absence or presence of MTGase. S: Surimi; M: MTGase (1.2 unit/g surimi); BG: Bovine gelatin; BFGM: Bovine/fish gelatin mix.

4.4.6 Likeness score of surimi gel added with gelatins in combination with and without MTGase

Likeness score of surimi gels added without and with gelatin in the presence and absence of MTGase is shown in Table 10. There was difference in likeness score for texture and overall between the control surimi gel and the gel added with MTGase (without 10% BG addition). Surimi gel added with MTGase had the rubbery texture as indicated by the increased breaking force and deformation (Figure 9). The excessive formation of ε-(γ-glutamyl) lysine crosslinks would yield the gel

with rigid and tough texture. No difference in color and appearance likeness between samples containing 10% BG and the control gel ($p>0.05$). The decrease in likeness of all attributes was observed as BFGM with FG/BG ratio increased ($p<0.05$). The lower texture and overall likeness score was probably due to the dilution effect of fish gelatin on myofibrillar proteins, especially when FG at higher proportion was present. The result indicated interfering effect of fish skin gelatin on surimi gel property as well as sensory property. However, gel added with MTGase and BFGM, having FG/BG ratio of 1:1 and 1:2, had the similar texture and overall likeness when compared with the control gel (without gelatin and MTGase). Thus, BFGM having the appropriate FG/BG ratio could be added into surimi along with MTGase to yield the gel with acceptability.

4.4.7 Microstructures of surimi gel added with gelatins in combination with and without MTGase

Microstructures of surimi gels added without and with MTGase in the present of 10% BG or BFGM at different BG/FG ratios are illustrated in Figure 11. Surimi gel network became finer with the addition of MTGase, as compared with the control gel (without MTGase). Those myofibrillar proteins could undergo the aggregation more effectively in the presence of MTGase. As a consequence, the more compact and dense gel network was developed. The result was in accordance with the higher breaking force and deformation (Figure 9) and the lowered expressible moisture content (Table 8) when MTGase was added in surimi. The coarser structure with a larger void was obtained when 10% BG or BFGM was added, though MTGase was combined. This confirmed the negative impact of fish gelatin on gelation of surimi. However, no difference in surimi gel microstructure was found when BFGM at different FG/BG ratios were used.

Table 9. Textural properties of surimi gel added 10% BG or BFGM without and with MTGase 1.2 units/g surimi

Samples	Hardness (N)*	Springiness (cm)*	Cohesiveness (ratio)*	Gumminess (N)*	Chewiness (N cm)*
Surimi (without gelatin and MTGase)	89.51±0.16^{b}	0.91±0.12^{b}	0.53±0.01^{a}	49.16±0.13^{b}	46.37±0.15^{a}
Surimi+MTGase (without gelatin)	98.97±0.13^{a}	0.98±0.37^{a}	0.60±0.00^{a}	57.49±0.14^{a}	49.67±0.22^{a}
Surimi+10% BG (without MTGase)	72.11±0.61^{e}	0.71±0.00^{d}	0.45±0.00^{c}	39.91±0.72^{d}	36.19±0.36^{b}
Surimi+10% BG with MTGase	88.86±0.42^{b}	0.91±0.01^{b}	0.56±0.00^{a}	47.90±0.22^{b}	45.79±0.11^{a}
Surimi+10% BFGM (the mixture of BG:FG, 1:1) with MTGase	86.69±0.78^{b}	0.86±0.00^{c}	0.51±0.05^{b}	43.81±0.18^{c}	40.18±0.15^{b}
Surimi+10% BFGM (the mixture of BG:FG, 2:1) with MTGase	87.03±0.61^{b}	0.88±0.00^{c}	0.51±0.51^{b}	45.00±0.72^{b}	44.19±0.36^{a}
Surimi+10% BFGM (the mixture of BG:FG, 1:2) with MTGase	83.90±0.42^{c}	0.86±0.01^{c}	0.50±020^{b}	42.90±0.22^{c}	39.79±0.11^{b}
Surimi+10% BFGM (the mixture of BG:FG, 4:1) with MTGase	87.42±0.47^{b}	0.88±0.01^{c}	0.52±0.00^{ab}	47.01±0.50^{b}	45.07±0.58^{a}
Surimi+10% BFGM (the mixture of BG:FG, 1:4) with MTGase	80.37±0.78^{d}	0.85±0.00^{c}	0.49±0.05^{b}	42.81±0.18^{c}	37.18±0.15^{b}

*Values are mean ± SD (n=3).

Different lowercase superscripts in the same column denote the significant differences (p<0.05).

BG: bovine gelatin; BFGM: bovine/fish gelatin mix; MTGase: microbial transglutaminase.

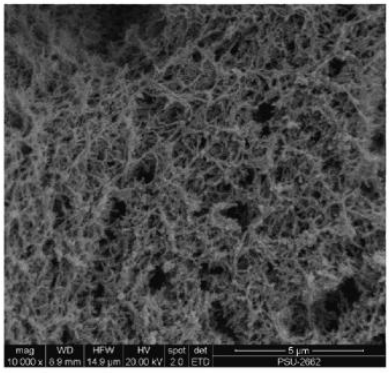
Table 10. Likeness score of surimi gel added 10% BG or BFGM without and with MTGase 1.2 units/g surimi

Samples	Likeness score*			
	Color	**Texture**	**Appearance**	**Overall**
Surimi (without gelatin and MTGase)	7.95±0.09^{a}	7.35±0.29^{a}	7.05±0.13^{a}	7.50±0.19^{a}
Surimi+MTGase (without gelatin)	7.93±0.51^{a}	5.47±0.84^{b}	7.30±0.02^{a}	6.17±0.81^{b}
Surimi+10% BG (without MTGase)	7.63±0.33^{a}	6.37±0.66^{ab}	6.86±0.35^{b}	6.50±0.10^{b}
Surimi+10% BG with MTGase	7.63±0.09^{a}	7.30±0.29^{a}	7.40±0.13^{a}	7.41±0.19^{a}
Surimi+10% BFGM (the mixture of BG:FG, 1:1) with MTGase	7.73±0.33^{a}	7.35±0.66^{a}	7.60±0.35^{a}	7.46±0.10^{a}
Surimi+10% BFGM (the mixture of BG:FG, 2:1) with MTGase	7.93±0.63^{a}	7.17±0.84^{a}	7.63±0.02^{a}	7.57±0.81^{a}
Surimi+10% BFGM (the mixture of BG:FG, 1:2) with MTGase	7.93±0.33^{a}	6.57±0.66^{ab}	7.55±0.35^{a}	6.57±1.00^{b}
Surimi+10% BFGM (the mixture of BG:FG, 4:1) with MTGase	7.72±0.46^{a}	6.80±0.64^{ab}	7.16±0.23^{a}	6.90±0.37^{b}
Surimi+10% BFGM (the mixture of BG:FG, 1:4) with MTGase	7.86±0.99^{a}	6.90±0.47^{ab}	6.43±0.22^{b}	6.85±0.20^{b}

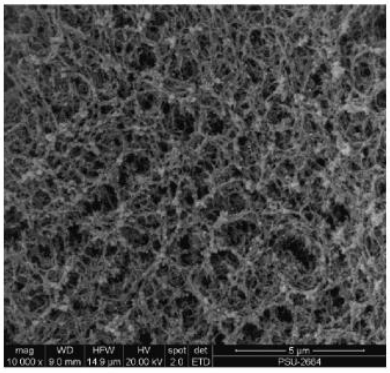
*Values are mean ± SD (n=3).

Different lowercase superscripts in the same column denote the significant differences (p<0.05).

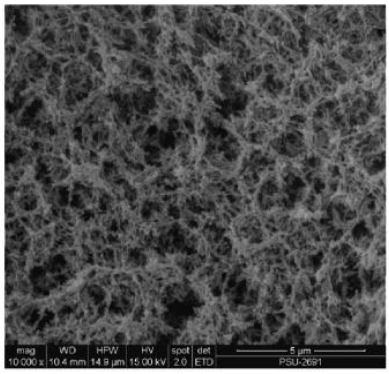
BG: bovine gelatin; BFGM: bovine/fish gelatin mix; MTGase: microbial transglutaminase.



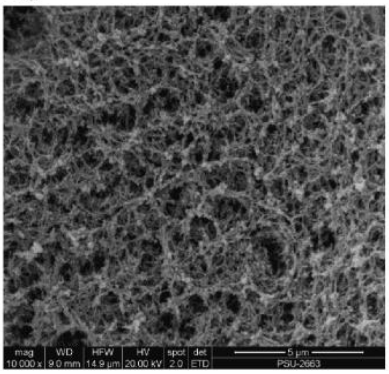
Surimi (without gelatin and MTGase)



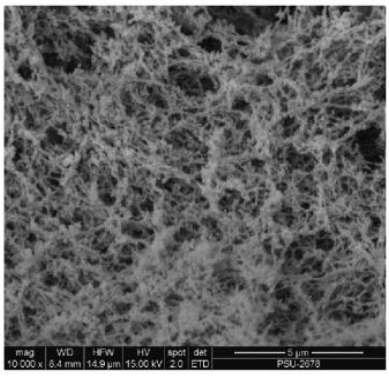
Surimi (without gelatin)+MTGase (1.2 units/g surimi)



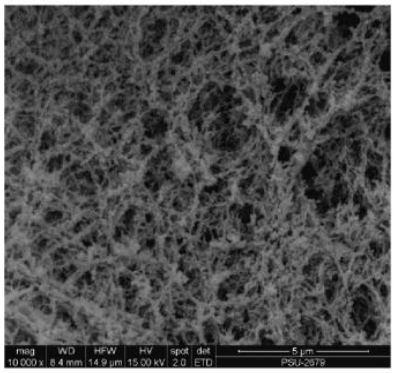
Surimi+10% BG (without MTGase)



Surimi+10% BG with MTGase (1.2 units/g surimi)



Surimi+10% BFGM (the mixture of BG and FG, 1:1) with MTGase (1.2 units/g surimi)



Surimi+10% BFGM (the mixture of BG and FG, 2:1) with MTGase (1.2 units/g surimi)

Figure 11. Electron microscopic images of surimi gel added with 10% BG (bovine gelatin) or BFGM (bovine/fish gelatin mix) without and with MTGase 1.2 units/g surimi (Magnification: 10,000X).

4.5 Conclusion

The addition of 10% bovine gelatin or bovine/fish gelatin mix in combination with MTGase into surimi directly affected the property of surimi gel. Surimi gel containing BG or BFGM with FG/BG ratio of 1:1 or 1:2 in conjunction with MTGase 1.2 units/g surimi could render the gel with acceptability equivalent to the control gel. Thus, gelatin at an appropriate level could be used as a source of collagen derivative in surimi with satisfactory property when MTGase was incorporated.

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CHAPTER 5

EFFECT OF UNICORN LEATHERJACKET (*ALUTERUS MONOCEROS*) GELATIN IN COMBINATION WITH MICROBIAL TRANSGLUTAMINASE ON GEL PROPERTIES OF THREADFIN BREAM SURIMI

5.1 Abstract

Gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) (UG) was extracted using the distilled water at different temperatures (50, 60, 70 and 80°C). The absorption bands of UG sample in FTIR spectra were mainly situated in the amide band and revealed the significant loss of molecular order of triple helix. UG had the decrease in gel strength as extraction temperature increased (p<0.05). Generally, gel strength of all UG samples was lower, compared with that of commercial bovine and fish gelatins (p<0.05). When surimi from threadfin bream (*Nemipterus bleekeri*) was added with UG and commercial fish gelatin (FG) at 10% protein substitution in combination with and without MTGase at 1.2 units/g surimi, breaking force and deformation of surimi gel decreased when the both gelatins were added (p<0.05). When MTGase was incorporated, the increases in breaking force and deformation of surimi gel were obtained. Addition of UG or FG generally lowered the expressible moisture content and whiteness of surimi gel (p<0.05). Based on SDS-PAGE, band intensity of MHC of surimi gel decreased when surimi gel was added with gelatins or MTGase. The microstructure study revealed that surimi gel network became finer and denser with the addition of MTGase at 1.2 units/g surimi, but the coarser and irregular structure was obtained when gelatin was incorporated.

5.2 Introduction

Gelatin is a polypeptide derived by hydrolytic degradation of collagen, which is widely found in nature as the major constituent of skin, bones and connective tissue (Park *et al.*, 2008). Gelatin has been applied in the food, pharmaceutical, medical, cosmetic and photographic industries (Bigi *et al.*, 2000; Pranoto *et al.*, 2007). Due to the outbreak of bovine spongiform encephalopathy (BSE) of bovine gelatin and prohibition of porcine gelatin by Muslim (Karim and Bhat, 2009), fish gelatin has gained increasing interests (Badii and Howell, 2006; Haug *et al.*, 2004). Fish gelatin can be produced from fish processing byproducts, e.g. skin, bone, scale, etc. (Kristinsson and Rasco, 2000; Sato *et al.*, 2003). In Thailand, unicorn leatherjacket is an economically important species commonly used for frozen fillet production. Thick skin of this species, generated during deskinning process, can serve as the promising raw material for gelatin extraction. Gelatin has served as the important ingradient in foods. With the addition of gelatin, surimi gel had the lower gel strength (Hernandez-Briones *et al.*, 2009). However, other protein additives have been widely used in surimi such as whey protein concentrate (Hsu and Sun, 2006; Rawdkuen and Benjakul, 2008; Youssef and Barbut, 2011), soy protein isolate (Luo *et al.*, 2004; Youssef and Barbut, 2011), egg white (Angela *et al.*, 2009; Duangmal and Taluengphol, 2010; Yetim and Ockerman, 1995), etc. Furthermore, microbial transglutaminase (MTGase) is another tool for textural improvement of several foods (Motoki and Seguro, 1998), particularly surimi products (Benjakul and Visessanguan, 2003; Benjakul *et al.*, 2004b; Chanarat *et al.*, 2012; Duangmal and Taluengphol, 2010). The use of MTGase in surimi containing fish gelatin is therefore a promising approach to strengthen the resulting gel.

However, no information regarding the effect of MTGase on the properties of surimi gel added with fish gelatin has been reported. Therefore, this study aimed to investigate the impact of gelatin from unicorn leatherjacket skin on the textural and physical properties of surimi gel from threadfin bream and the effect of MTGase on the properties of surimi gel containing fish gelatin.

5.3 Materials and methods

5.3.1 Chemicals/Gelatin

Sodium dodecyl sulfate (SDS), β-mercaptoethanol (βME), glycerol, high molecular weight marker 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). Phosphoric acid was obtained from Merck (Darmstadt, Germany). Bovine hide gelatin and fish gelatin were purchased from Halamic company (Bangkok, Thailand) and LAPI GELATINE S.p.A. (Empoli, Italy), respectively. Microbial transglutaminase (MTGase) from *Streptoverticillium mobaraense* (TG-K) with actively of 100 units/g dry matter was supplied by Ajinomoto (Thailand) Co., Ltd. (Bangkok, Thailand). This enzyme powder consisted of 99% maltodextrin and 1% enzyme.

5.3.2 Extraction of gelatin from the skins of unicorn leatherjacket (*A. monoceros*)

5.3.2.1 Fish skin preparation

The skin of unicorn leatherjacket (*A. monoceros*) was obtained from Sea Wealth Frozen Food Co., Ltd., Songkhla, Thailand. Upon arrival to the Department of Food Technology, Prince of Songkla University, Hat Yai, the skin was cleaned and washed with iced tap water (0-2°C). Prepared skin was then cut into small pieces (0.5×0.5 cm^2), placed in polyethylene bags and stored at -20°C until use. The storage time was less than 2 months.

5.3.2.2 Extraction of gelatin from the skin of unicorn leatherjacket

Before gelatin extraction, the prepared skin was soaked in 0.05 M NaOH with a skin/solution ratio of 1:10 (w/v). The mixture was stirred continuously for 2 h at room temperature at a speed of 150 rpm using an overhead stirrer equipped with propeller (RW 20.n, IKA®Werke GmbH & CO.KG, Staufen, Germany). The alkaline solution was changed every 1 h to remove non collagenous proteins and

pigments. Alkaline-treated skin was then washed with tap water until neutral or faintly basic pH of wash water was obtained. The skin was then soaked in 0.1 M phosphoric acid with a skin/solution ratio of 1:10 (w/v) for 24 h with gentle stirring at room temperature. The acidic solution was changed every 12 h to swell the collagenous material in the fish skin matrix. Acid-pretreated skin was washed thoroughly with tap water until wash water became neutral or faintly basic. To extract gelatin, the swollen skin was soaked in distilled water at different temperatures (50, 60, 70 and 80°C) with a skin/water ratio of 1:10 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 12 h with a continuous stirring at a speed of 150 rpm. The mixture was then filtered using two layers of cheesecloth. The filtrate was further filtered using a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England) with the aid of JEIO Model VE-11 electric aspirator (JEIO TECH, Korea). The resulting filtrate was freeze-dried using a ScanvacModel Coolsafe 55 freeze dryer (Coolsafe, Lynge, Denmark).

5.3.2.3 Yield and characterization

5.3.2.3.1 Determination of yield

Gelatin yield was calculated by the following equation:

Yield% = [weight of dry gelatin (g)/weight of wet unicorn leatherjacket skin (g)]×100

5.3.2.3.2 Determination of gel strength

Gels of gelatin were prepared by the method of Fernández-Díaz *et al.* (2001) with a slight modification. Gelatin samples were dissolved in distilled water at 60°C to obtain the final concentration of 6.67% (w/v). The solution was stirred until the gelatin was solubilized completely and cooled in a refrigerator at 10°C for 16-18 h for gel maturation. The dimensions of the sample were 3 cm in diameter and 2.5 cm in height. The gel strength was determined using a Model TA-XT2 Texture Analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kN and equipped with a 1.27 cm diameter flat faced cylindrical Teflon® plunger. The maximum force (in grams) was recorded when the penetration distance reached 4 mm. The speed of the plunger was 0.5 mm/s.

5.3.2.3.3 Color measurement

The color of gelatin gels (6.67% w/v) was measured by a Hunter lab color meter (Color Flex, Hunter Lab Inc., Reston, VA, USA) and was reported by the CIE system. L*, a* and b* parameters indicate lightness/brightness, redness/greenness and yellowness/blueness, respectively. The colorimeter was warmed up for 10 min and calibrated with a white standard.

5.3.2.3.4 Electrophoretic analysis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970). Gelatin samples were dissolved in 5% SDS and the mixtures were incubated at 85°C for 1 h. The mixtures were centrifuged at 5,000 g for 5 min at room temperature using a microcentrifuge (MIK-RO20, Hettich Zentrifugan, Germany) to remove undissolved debris. Protein concentration of the supernatant was determided by the Biuret method (Robinson and Hodgen, 1940). Gelatin samples were mixed at a 1:1 (v/v) ratio with the sample buffer (0.5 M Tris-HCl, pH 6.8, containing 4% SDS and 20% glycerol). Samples (l5 µg protein) were loaded onto polyacrylamide gels comprising a 7.5% running gel and a 4% stacking gel and subjected to electrophoresis at a constant current of 15 mA/gel using a Mini Protein II unit (Bio-Rad Laboratories, Inc., Richmond, CA, USA). After electrophoresis, the gel was stained with 0.05% (w/v) Coomassie Blue R- 250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid. High molecular weight markers (GE Healthcare UK, Buckinghamshire, UK) were used to estimate the molecular weight of the proteins. Food grade bovine gelatin was used as a standard gelatin.

5.3.2.3.5 Fourier transform infrared (FTIR) analysis

FTIR spectra of freeze-dried gelatin samples were recorded using a horizontal ATR Trough plate crystal cell (45° ZnSe; 80 mm long, 10 mm wide and 4 mm thick) (PIKE Technology Inc., Madison, WI, USA) equipped with a Bruker Model Equinox 55 FTIR spectrometer (Bruker Co., Ettlingen, Germany) at room temperature. For spectra analysis, freeze-dried gelatin samples were placed on the

crystal cell and the cell was clamped into the mount of the FTIR spectrometer. The spectra in the range of 400-4000 cm^{-1} were ratioed and automatic signals gained were collected in 32 scans at a resolution of 4 cm^{-1} against a background spectrum recorded from the clean empty cell at 25°C.

5.3.3 Preparation of surimi gel added with unicorn leatherjacket gelatin and MTGase

Frozen surimi from threadfin bream (*Nemipterus bleekeri*) was purchased from Pacific Fish Processing Co., Ltd., Songkhla, Thailand. Frozen surimi was partially thawed at 4°C for 8-10 h to obtain the core temperature of approximately 0-2°C. Surimi was then cut into small pieces and mixed with 2.5% NaCl in a mixer (MK-5087M, Panasonic Manufacturing Malaysia Berhad, Selangor, Malaysia). During chopping, the temperature was maintained below 10°C. Gelatin from skin of unicorn leatherjacket (UG) extracted at the temperature yielding the highest gel strength or commercial fish gelatin (FG) was then added into surimi paste at 10% protein substitution. The mixtures were chopped for 5 min. To the mixture, MTGase was then added to obtain the level of 1.2unit/g 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.

5.3.3.1 Determination of surimi gel properties

5.3.3.1.1 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 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) until the puncture occurred. The force applied (breaking force) and the distance at which the probe punctured into the gel (deformation) were both recorded.

5.3.3.1.2 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) (Bourne, 1978) using a cylinder probe with a diameter of 2.5 cm. Hardness, springiness, cohesiveness, gumminess and chewiness were determined.

5.3.3.1.3 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:

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

5.3.3.1.4 Whiteness

Whiteness of gel samples was determined as described by Benjakul *et al.* (2004a) using a colorimeter (model ColorFlex, HunterLab Reston, VA, USA.). 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

5.3.3.1.5 Electrophoretic analysis

Protein patterns of surimi paste and different 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 (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 3,500 xg for 20 min to remove undissolved debris. The samples (15 µg protein) were loaded onto the polyacrylamide gel made of 10% running gel and 4% stacking gel and subjected to electrophoresis. After separation, the proteins were stained and destained as mentioned previously.

5.3.3.1.6 Microstructure

Microstructure of surimi gel samples added without and with gelatin in the presence and absence of MTGase (1.2 units/g surimi) was determined using scanning electron microscope (SEM). Gel samples were cut into small pieces (0.25x0.25x0.25 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).

5.3.4 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).

5.4 Results and discussion

5.4.1 Effect of different extraction temperatures on yield and properties of gelatin from unicorn leatherjacket skin

5.4.1.1 Extraction yield of gelatin extracted with different temperatures

Yield of gelatin from the skins of unicorn leatherjacket extracted at 50, 60, 70 and 80°C were 2.54, 3.17, 4.92 and 6.83% (wet weight basis), respectively. With higher extraction temperature, the higher yield of gelatin was obtained. The result was in accordance with Ward and Courts (1977) who reported that the yield of gelatin from fish skin extracted at 50°C was much higher than that extracted at 40°C. Several factors (liming concentrations, extraction solution pH, extraction temperature and extraction time) were shown to affect the extraction yield of gelatin from skate (*Raja Kenojei*) skin (Cho *et al*., 2006). Yields of gelatin extracted from skate skins increased with increasing extraction temperature (Cho *et al.*, 2006). In addition, Kittiphattanabawon *et al.* (2010) reported that the yield of gelatin from the skins of brownbanded bamboo shark and blacktip shark increased with increasing extraction temperatures and times. Moreover, Arnesen and Gildberg (2007) and Muyonga *et al.* (2004) reported that the increasing extraction temperatures and times resulted in the increased yield of gelatin from Atlantic salmon skin as well as Nile perch skin and bone. Thus, extraction temperature determined the yield of gelatin from the skin of unicorn leatherjacket.

5.4.1.2 Gel strength

Gel strength of gelatin gels from unicorn leatherjacket extracted at various temperatures (50, 60, 70 and 80°C) is shown in Figure 12. Gel strength of gelatin gels from unicorn leatherjacket skin decreased as the extraction temperature increased ($p<0.05$). Muyonga *et al.* (2004) also reported that gelatin extracted from Nile perch (*Lates niloticus*) skin and bone at higher temperatures exhibited lower gel strength. Similarly, gelatin extracted from dorsal skin of yellowfin tuna (*Thunnus albacares*) had the higher gel strength when the lower extraction temperature was used (Cho *et al.*, 2005). Gel forming ability and the physical properties of gelatin

from skate skin decreased as extraction temperature increased (Cho *et al.*, 2006). Gelation occurs via the aggregation of protein molecules, leading to the formation of junction zones and ultimately a three-dimensional branched network (Gilsenan and Ross-Murphy, 2000). Kittiphattanabawon *et al.* (2010) reported that gelatins from the skins of brownbanded bamboo shark and blacktip shark skins showed the decreases in gel strength as the extraction temperature and time increased. Gelatin, with the lower chain length, generally could not form the strong gel due to the lower inter-junction zones (Benjakul *et al.*, 2009). Therefore, extraction temperature directly affected gelling property of gelatin from the skin of unicorn leatherjacket.

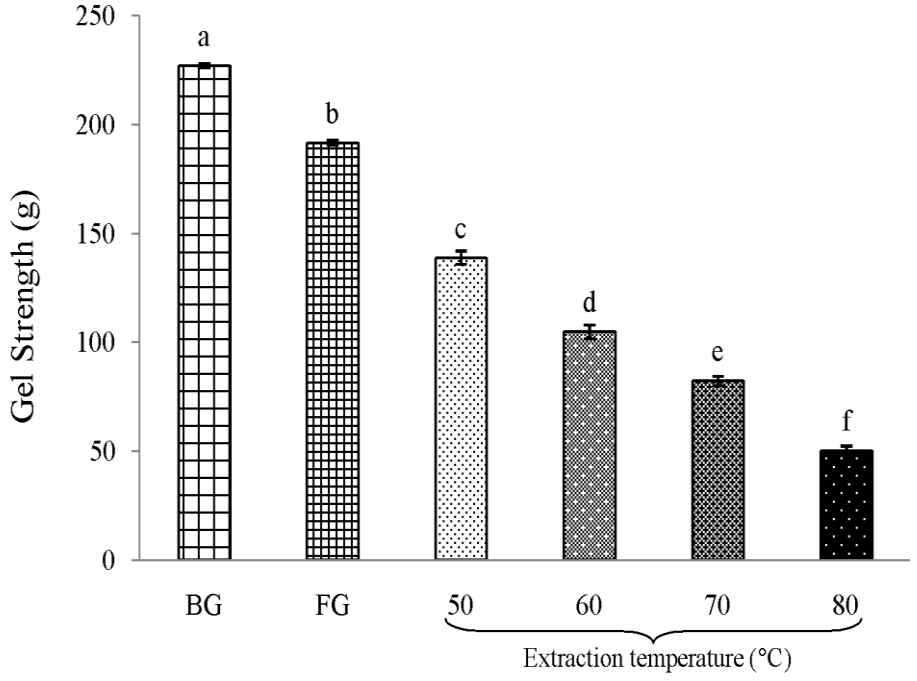

Figure12. Gel strength of gelatin from unicorn leatherjacket skin extracted at different temperatures FG: fish gelatin, BG: bovine gelatin. Bars represent the standard deviation (n:3). Different letters on the bars indicate significant difference (p<0.05).

5.4.1.3 Color

The color of gel gelatin from unicorn leatherjacket skin extracted at different temperatures is presented in Table 11. Gelatin manufactures generally have a good method to clarify the impurities from the gelatin solution, such as chemical

clarification and filtration processes. Generally, the color did not affect functional properties of gelatin (Ockerman and Hansen, 1988). A higher L*-value of gelatin gel was observed as higher temperatures was used (p<0.05). The higher b*-value (yellowness) and a*-value (redness) of gelatin were found with increasing extraction temperatures. Gelatin gel from unicorn leatherjacket skin had lower lightness than the commercial fish gelatin. This was probably due to the differences in raw material and extraction conditions used between those extracted from unicorn leatherjacket skin and the commercial counterpart.

Table 11. Color of gelatin from the skin of unicorn leatherjacket with different extraction temperatures

Samples	L*	a*	b*
Commercial bovine gelatin	36.65±0.09^{b}	-0.28±0.08^{c}	-1.76±0.04^{f}
Commercial fish gelatin	41.01±0.05^{a}	0.37±0.14^{a}	2.51±0.02^{a}
50°C	22.03±0.02^{f}	-1.17±0.13^{e}	-0.90±0.05^{d}
60°C	24.52±0.04^{e}	-0.71±0.11^{d}	-1.22±0.07^{e}
70°C	28.29±0.28^{d}	0.29±0.04^{b}	2.16±0.08^{c}
80°C	33.49±0.51^{c}	0.36±0.04^{a}	2.32±0.05^{b}

Numbers 50°C, 60°C, 70°C and 80°C indicate extraction temperatures.
Different lowercase superscripts in the same column denote significant difference (p<0.05).

5.4.1.4 Protein patterns

Protein patterns of gelatin from the skins of unicorn leatherjacket extracted at different temperatures are shown in Figure 13. For gelatin extracted at 50°C, β- and α-chains appeared as the major components. Nevertheless, the decrease in band intensity of those major components in gelatins was noticeable when the extraction was performed at higher temperatures. The proteins or peptides with a molecular weight lower than the α-chain were found in gelatins extracted at higher temperatures. This might be caused by the more pronounced degradation induced by

the thermal process during gelatin extraction. The appearance of low molecular weight components in gelatin suggested that some hydrolysis took place in gelatin during extraction process. For the gelatin samples extracted at lower temperature, proteins with MW greater than β-chain were also found as appeared in the stacking gel. Proteins with MW greater than β-chain had a high positive correlation with gel strength (Kittiphattanabawon *et al.*, 2010; Muyonga *et al.*, 2004). Kittiphattanabawon *et al.* (2010) reported that the band intensity of β-, γ- and α-chains of gelatin from brownbanded bamboo shark and blacktip shark skins decreased with increasing extraction temperatures and times. When gelatin was extracted at 75°C, no α-, β- and γ-chains were retained. Furthermore, Muyonga *et al.* (2004) revealed that Nile perch skin gelatins contained lower MW peptides, with MW less than α-chain, especially when extracted at higher temperature. The result indicated that extraction at temperature above 60°C negatively affected the gel strength of gelatin from unicorn leatherjacket skin. The shorter chain peptides or fragments of gelatin could not form the junction zone. As a result, the weak network could be developed as evidenced by the lowered gel strength. Since gelatin from unicorn leatherjacket skin extracted at 50°C rendered the highest gel strength, it was further used for addition in surimi.

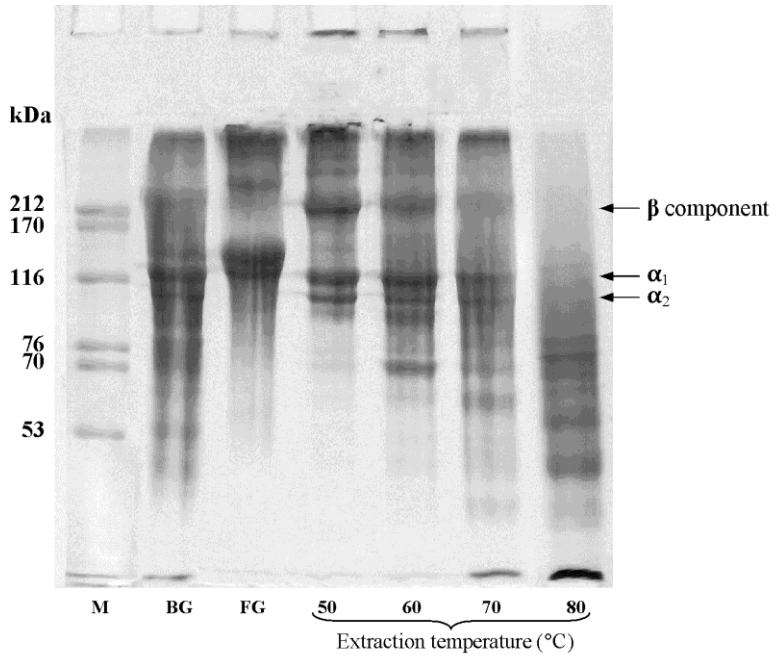

Figure 13. Protein pattern of gelatin from the skin of unicorn leatherjacket extracted at different temperatures. Numbers 50°C, 60°C, 70°C and 80°C indicate extraction temperatures.

5.4.1.5 Fourier transform infrared (FTIR) spectra

FTIR spectra of gelatin from the skin of unicorn leatherjacket extracted at various temperatures are depicted in Figure 14. Fourier transform infrared (FTIR) spectroscopy has been used to study the changes in the secondary structure of gelatin (Muyonga *et al.*, 2004). Gelatin from the skins of unicorn leatherjacket skin extracted at 50, 60, 70 and 80°C exhibited the amide A bands at the wavenumber of 3278.14, 3284.73, 3300.16 and 3302.08 cm^{-1}, respectively. The gelatin extracted at higher temperature had the shift of amide A band to the higher wavenumber, compared with those extracted at lower temperatures. Gelatin extracted at high temperature consisted mainly of low molecular weight peptides. The shift of amide A band arose from the stretching vibrations of N-H group (Ahmad and Benjakul, 2011). Amide A tends to merge with the CH_2 stretch peak when carboxylic acid groups exist in stable dimeric (intermolecular) associations (Kemp, 1987; Muyonga *et al.*, 2004).

Gelatin from the skin of unicorn leatherjacket extracted at 50, 60, 70 and 80°C exhibited the amide I bands at the wavenumber of 1689.61, 1699.62, 1691.54 and 1647.18 cm^{-1}, respectively. Gelatin from the skin of unicorn leatherjacket extracted at 50, 60, 70 and 80°C exhibited the amide II bands at the wavenumber of 1544.95, 1550.74, 1546.88 and 1539.17 cm^{-1}, respectively. Amide I and amide II bands of gelatins at 1600-1700 and 1500-1560 cm^{-1} were reported by Muyonga *et al.* (2004), Yakimets *et al.* (2005), Ahmad and Benjakul (2011). The amide I vibration mode is primarily a C=O stretching vibration coupled to contributions from the CN stretch, CCN deformation and in-plane NH bending modes (Ahmad and Benjakul, 2011; Bandekar, 1992; Lavialle *et al.*, 1982; Muyonga *et al.*, 2004). The absorption in the amide I region is the most useful for infrared spectroscopic analysis of the secondary structure of proteins (Surewicz and Mantsch, 1988). Yakimets *et al.* (2005) reported that the absorption peak at 1633 cm^{-1} was characteristic for the coil structure of gelatin. The amide II vibration mode is attributed to an out-of-phase combination of CN stretch and inplane NH deformation modes of the peptide group (Bandekar, 1992; Lavialle *et al.*, 1982). The amide II band is generally considered to be much more sensitive to hydration than to secondary structure change (Wellner *et al.*, 1996). It is well known that the method of manufacture affects the physicochemical

properties of gelatin. Covalent cross-links between α-chains, hydrogen bonds that stabilize the triple helix and even peptide bonds in the primary structure are ruptured during these treatments (Hinterwaldner, 1977; Ledward and J.R. Mitchell, 1986; Veis, 1964). From the result, gelatin extracted at 80°C had the lower wavenumbers for both amide I and amide II, compared with others. The result suggested that carbonyl group or NH_2 group of peptides in this sample underwent interaction with their reactive groups, plausibly via Maillard reaction. This was evidenced by the increased yellow color of gelatin extracted at higher temperatures. Thus, it can be concluded that the secondary structure of gelatins obtained from the skin of unicorn leatherjacket was affected by extraction temperature.

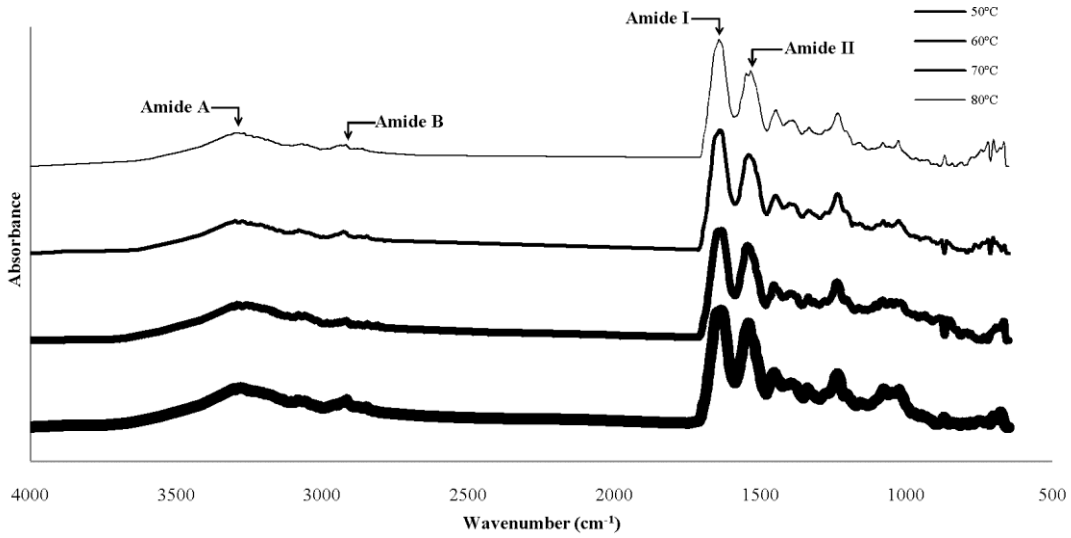

Figure 14. Fourier transform infrared (FTIR) spectra of gelatin from the skin of unicorn leatherjacket extracted at different temperatures.

5.4.2 Properties of surimi gel added with gelatins in combination without and with MTGase

5.4.2.1 Breaking force and deformation

Breaking force and deformation of surimi gel from threadfin bream added with 10% gelatin (FG or UG) in the presence or absence of MTGase are shown in Figure 15. Without MTGase addition, breaking force and deformation of surimi gel decreased as both gelatins (FG/UG) were added (p<0.05). This was more likely due

to the dilution effect of gelatin on myofibrillar proteins in surimi. Surimi gel added with 10% UG and 10% FG had the decreases in breaking force by 32.21 and 27.43% respectively, compared with the control gel. UG showed slightly more negative effect on surimi gel property than FG, as evidenced by the slightly lower breaking force ($p<0.05$). However, similar deformation was found between surimi gel added with UG and FG ($p<0.05$). As above mentioned, UG had the lower gel strength, compared with FG. It was more likely due to the shorter chains of UG. Those short chains could not form the strong network. Various proteins and carbohydrates such as whey protein concentrate, wheat gluten (Chen, 2000; Murphy *et al.*, 2005) alginates, xanthan and high methoxyl pectins have a negative effect on surimi and fish mince gels (Barrera *et al.*, 2002; Park, 2000). Moreover, the detrimental effect on mechanical properties of surimi gels might be associated with disruptive effect of gelatin on the formation of three-dimensional structure of myofibrillar proteins (Hernandez-Briones *et al.*, 2009). 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 fish gelatin at level of 15 g/kg and 7.5 g/kg was added, respectively.

When MTGase was incorporated into surimi (without gelatin), breaking force and deformation increased by 32.09 and 25.24%, compared with those of the control ($p<0.05$). Therefore, MTGase was effective in increasing the gel strength of surimi. For surimi added with 10% UG or FG, the addition of MTGase also increased breaking force and deformation, compared with that containing 10% UG or FG (without MTGase). MTGases is an enzyme that catalyzes the cross-linking of proteins through the formation of covalent bonds between protein molecules. MTGase could increase breaking force and deformation of surimi containing 10% UG by 73.92 and 26.09%, respectively. The increases in breaking force and deformation by 70.64 and 23.43%, respectively were found with the addition of 10% FG with MTGase.

The effect of MTGase at different levels (0 to 0.8 units/g 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 was studied (Benjakul *et al.*, 2008). MTGase showed the gel strengthening effect on lizardfish mince, particularly when high amounts of MTGase were used. The addition of MTGase is reported to

induce the cross-linking of MHC, thereby increasing the gel strength (Hsieh *et al.*, 2002; Jiang *et al.*, 2000; Sakamoto *et al.*, 2006). The addition of MTGase in surimi containing 10% UG or FG was able to improve gel properties. MTGase could induce cross-linking of MHC, in which gelatin was distributed along with those cross-links.

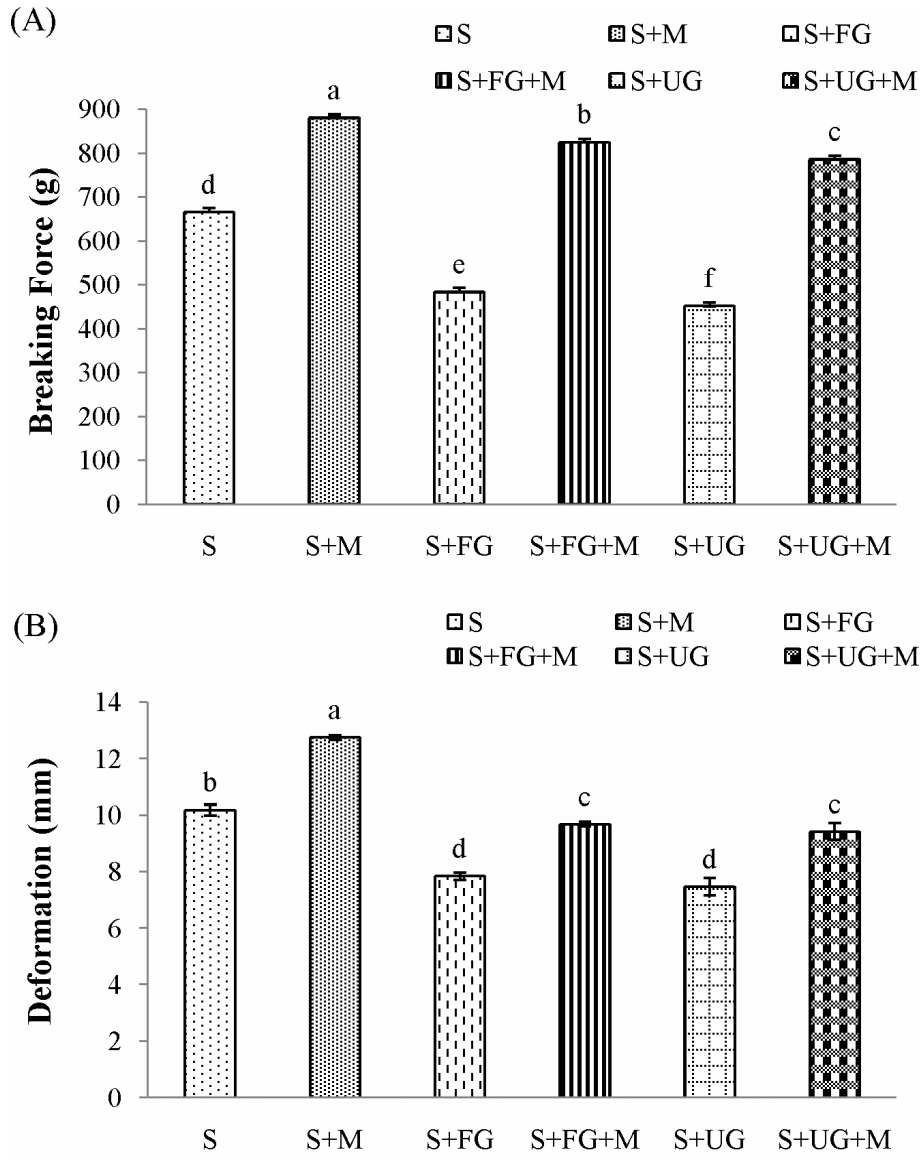

Figure 15. Breaking force (A) and deformation (B) of surimi gel added with gelatin (FG: commercial fish skin gelatin; UG: unicorn leatherjacket skin gelatin) in the presence and absence of MTGase at 1.2 units/g surimi (M). Different letters on the bar indicate the significant differences (p<0.05). Bars represent the standard deviations (n=3).

5.4.2.2 Expressible moisture content

Expressible moisture content of surimi gel added with UG and FG in the presence and absence of MTGase is shown in Table 12. The decreases in expressible moisture content were observed in surimi gels when both gelatins (UG/FG) were added ($p<0.05$). The result indicated that fish gelatin was hydrophilic in nature and could bind water via H-bond. Gelatin could hold water molecularly in gel matrix and improved water-holding capacity of surimi gel. According to Hernandez-Briones *et al.* (2009), gels from Alaska pollock surimi containing 7.5-15 g/kg of fish gelatin showed the improved water holding capacity.

When MTGase was incorporated into surimi, the decrease in expressible moisture content was found in surimi gel, regardless of gelatin incorporated ($p<0.05$). 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 surimi) in surimi from threadfin bream, Indian mackerel (*Rastrelliger kanagurta*) and sardine (*Sardinella gibbosa*) resulted in the decreases in expressible moisture content. Furthermore, surimi gel added with FG or UG in combination with MTGase had less expressible moisture content than surimi gel containing FG or UG (without MTGase) ($p<0.05$).

Addition of MTGase induced the inter-connection of gel matrix, thereby enhancing water holding capacity of gel (Han *et al.*, 2009; Moreno *et al.*, 2008). The result was in accordance with Trespalacios and Pla (2007) who reported that adding 0.3% MTGase on chicken meat gel significantly decreased expressible moisture content. The addition of 0.5% MTGase to beef homogenates significantly decreased expressible moisture, cooking yield and purge loss from beef gels (Pietrasik, 2003). When both gelatin and MTGase were added in surimi gel, the water holding capacity was markedly increased. The decrease in expressible moisture content was in accordance with higher breaking force and deformation (Figure 15). Thus, MTGase and gelatin addition showed the combined effect on water holding capacity of gel.

5.4.2.3 Whiteness

Slight decreases in whiteness were found in surimi gels when UG was added, regardless of MTGase incorporation ($p<0.05$) (Table 12). The decrease in whiteness might be due to brownish yellower color of UG. Rawdkuen *et al.* (2004) reported that gel of surimi from lizardfish had the decreased whiteness when chicken plasma protein was added. There was no difference in whiteness between surimi gels added with MTGase and the control gel ($p>0.05$). Therefore, MTGase addition had no impact on whiteness of resulting surimi gels. Whiteness is another quality index of surimi gel. The additives have been reported to affect the whiteness of surimi gel, depending on type and amount of additives incorporated (Benjakul and Visessanguan, 2000; Benjakul *et al.*, 2001; Benjakul *et al.*, 2007). At a level of 10% gelatin, the whiteness of surimi from threadfin bream was not affected, however it depended on the source of gelatin added.

Table 12. Expressible moisture content and whiteness of surimi gel added with 10% FG or 10% UG in the presence or absence of MTGase at 1.2 units/g surimi

Samples	Expressible moisture content (%)*	Whiteness*
Surimi (without gelatin and MTGase)	2.95±0.04[a]	81.52±0.63[a]
Surimi+ MTGase (without gelatin)	2.56±0.05[b]	80.47±0.46[a]
Surimi+10% FG	2.15±0.07[c]	79.81±0.10[a]
Surimi+10% FG with MTGase	1.74±0.08[d]	79.71±0.23[a]
Surimi+10% UG	2.35±0.05[c]	78.63±0.09[b]
Surimi+10% UG with MTGase	1.98±0.09[d]	78.59±0.27[b]

*Values are mean ± SD (n=3).

Different lowercase superscripts in the same column denote the significant differences ($p<0.05$). FG: commercial fish skin gelatin; UG: unicorn leatherjacket skin gelatin; MTGase: microbial transglutaminase.

5.4.2.4 Protein patterns

Protein patterns of surimi gels added without and with gelatin (UG or FG) in the presence or absence of MTGase are depicted in Figure 16. Slight decrease in MHC band intensity was found in surimi gel when MTGase was incorporated, compared with that observed in control gel (without gelatin and MTGase). It was noted that the 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 disappearance of MHC was probably associated with the formation of ε-(γ-glutamyl) lysine intra and intermolecular cross-links of proteins induced by endogenous TGase. The improvement of gel strength in some tropical fish was reported to be achieved by setting at 40°C, and was associated with the increased cross-linking of MHC and formation ε-(γ-glutamyl) lysine isopeptide (Benjakul and Visessanguan, 2003; Benjakul *et al.*, 2004b). The addition of both UG and FG as a substituent resulted in the dilution of muscle proteins, which was a major contributor for gel formation. This was evidenced by the slight decrease in MHC in surimi gel. In general, the cross-linking induced by both endogenous TGase and MTGase occurred in gel added with MTGase as indicated by polymerized proteins. Nevertheless, no marked changes in actin band were found. 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 intensity. Therefore, MTGase could work synergistically with endogenous TGase in cross-linking of protein in surimi, regardless of gelatin addition.

5.4.2.5 Textural properties

TPA paremeters of surimi gels added without and with UG or FG in the presence and absence of MTGase are shown in Table 13. When UG or FG were added, hardness, springiness, cohesiveness, gumminess and chewiness decreased ($p<0.05$). The results indicated that gelatin might disturb the three-dimensional structure of myofibrillar protein networks. This was in agreement with the decrease in

breaking force and deformation of surimi gel (Figure 15). Slight increases in hardness, springiness and cohesiveness were observed in all samples containing 10% UG or FG and the control gel when MTGase was incorporated (p<0.05). However, surimi gels added with FG showed higher hardness and springiness than those added with UG, regardless of MTGase addition (without and with MTGase) (p<0.05). 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. Generally, the addition of fish gelatin resulted in the poorer textural properties of surimi gel. However, the addition of MTGase was able to improve the textural properties of surimi gel containing gelatin or mixed gelatins to some extent.

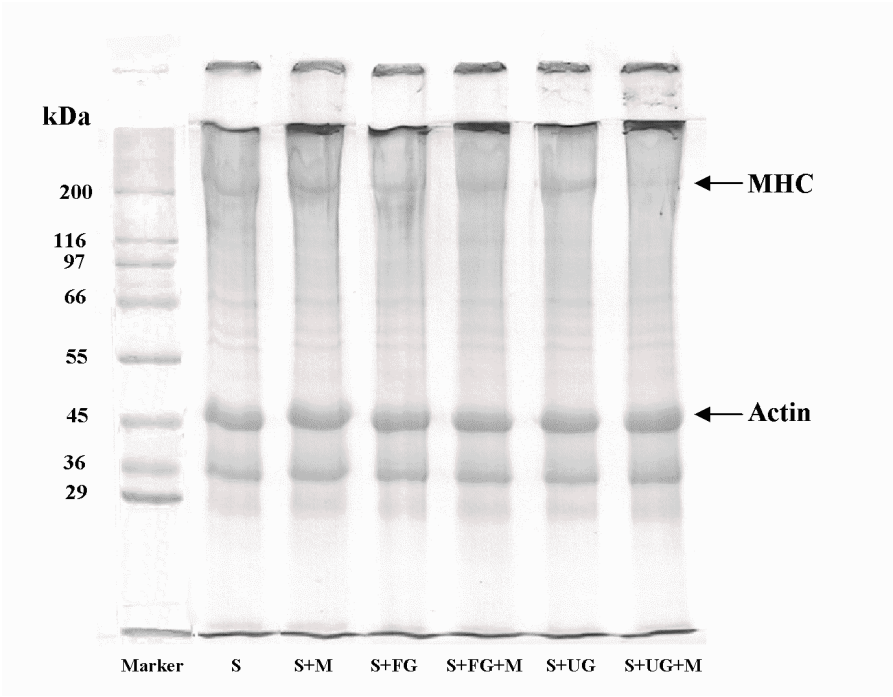

Figure 16. Protein pattern of surimi gel added with gelatin. Marker: markers; S: surimi paste; FG: commercial fish skin gelatin; UG: unicorn leatherjacket skin gelatin; M: MTGase: microbial transglutaminase.

5.4.2.6 Microstructures

Microstructures of surimi gels added without and with MTGase in the presence of 10% UG or FG are illustrated in Figure 17. Surimi gel network became finer and denser with the addition of MTGase, as compared with the control gel

(without MTGase). Those myofibrillar proteins could undergo the aggregation more effectively in the presence of MTGase. As a consequence, the more compact and dense gel network was developed. The result was in accordance with the higher breaking force and deformation (Figure 15) and the lowered expressible moisture content (Table 12) when MTGase was added in surimi. The coarser structure with a larger void was obtained when UG or FG were added, though MTGase was combined. Irregular structures with larger voids were in agreement with of poorer gel properties of surimi gel containing gelatin (UG/FG).

5.5 Conclusion

Gelatin from unicorn leatherjacket skins extracted at 50°C exhibited the highest gel strength, compared with those extracted at higher temperatures. The addition of 10% gelatin (UG or FG) in conjunction with MTGase at 1.2units/g surimi was recommended to obtain surimi with grade AA.

Table 13. Textural properties of surimi gel added with 10% FG or 10% UG in the presence or absence of MTGase at 1.2 units/g surimi

Samples	Hardness (N)*	Springiness (cm)*	Cohesiveness (ratio)*	Gumminess (N)*	Chewiness (N cm)*
Surimi (without gelatin and MTGase)	87.68±0.04^{b}	0.89±0.03^{b}	0.54±0.02^{c}	73.51±0.23^{b}	35.48±0.80^{b}
Surimi+MTGase (without gelatin)	99.54±0.94^{a}	0.99±0.05^{a}	0.62±0.01^{a}	86.77±0.39^{a}	58.54±0.72^{a}
Surimi+10% FG	71.40±0.28^{c}	0.73±0.01^{c}	0.52±0.02^{c}	51.54±0.56^{c}	32.70±0.31^{c}
Surimi+10% FG with MTGase	83.86±0.70^{b}	0.82±0.00^{b}	0.58±0.01^{b}	72.32±0.37^{b}	34.72±0.61^{c}
Surimi+10% UG	69.34±0.23^{c}	0.65±0.05^{d}	0.52±0.01^{c}	51.36±0.42^{c}	32.46±0.80^{c}
Surimi+10% UG with MTGase	80.61±0.50^{b}	0.76±0.05^{c}	0.57±0.01^{b}	70.49±0.29^{b}	34.15±0.21^{c}

*Values are mean ± SD (n=3).

Different lowercase superscripts in the same column denote the significant differences (p<0.05).

FG: commercial fish skin gelatin; UG: unicorn leatherjacket skin gelatin; MTGase: microbial transglutaminase.

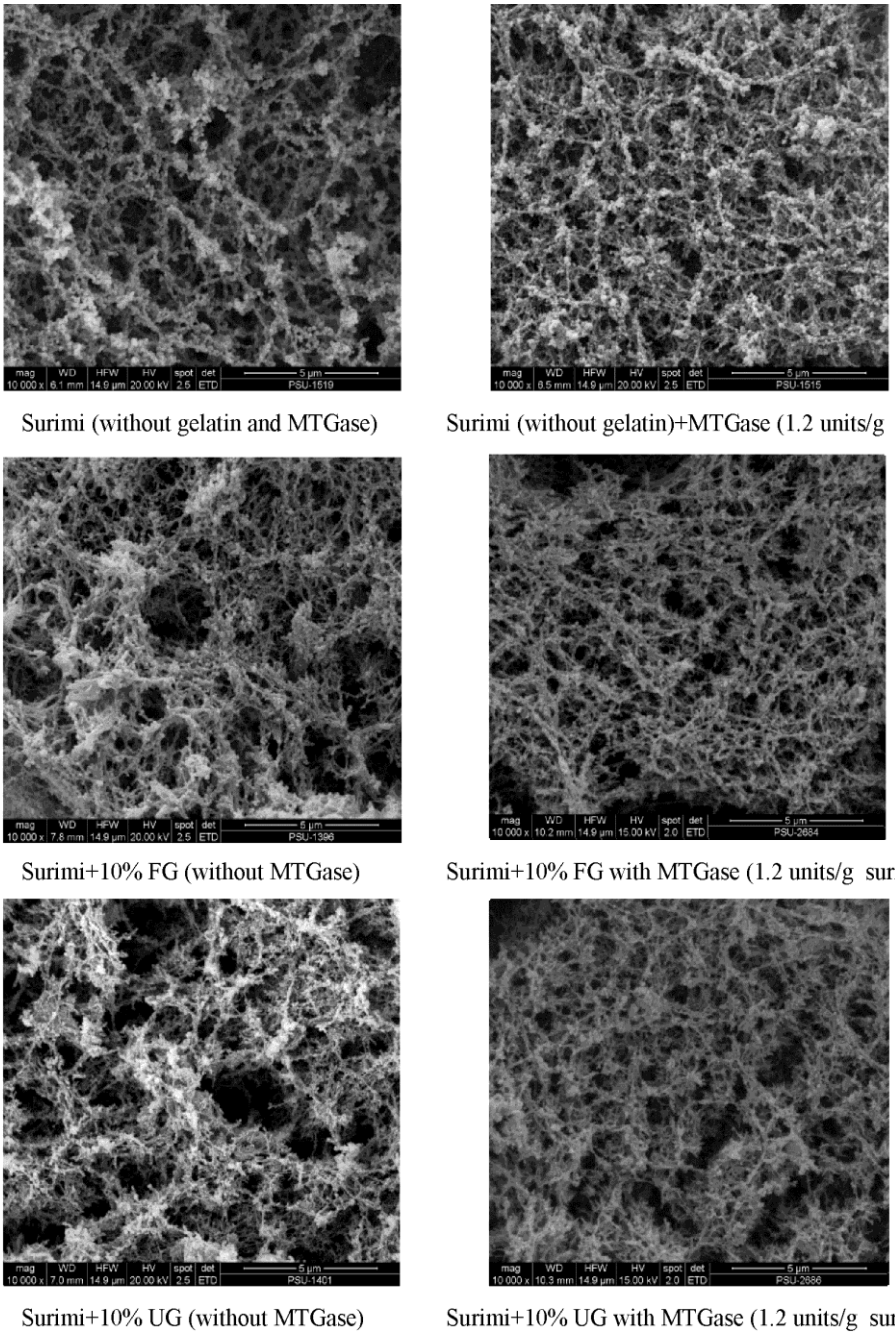

Figure 17. Electron microscopic images of surimi gel added with gelatin (Magnification: 10,000X). (FG: commercial fish skin gelatin; UG: unicorn leatherjacket skin gelatin) in the presence and absence of MTGase at 1.2 units/g surimi.

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CHAPTER 6

SUMMARY AND FUTURE WORKS

6.1 Summary

1. Fish gelatin was able to hold water in the surimi gel effectively as evidenced by the decrease in expressible moisture content. However, fish gelatin at high concentration had negative effect on mechanical properties of surimi gels.
2. OTA at an appropriate level could improve the property of surimi gel containing fish gelatin. Nevertheless, OTA addition resulted in the darker color. Addition of fish gelatin along with OTA generally increased the water holding capacity of surimi gel.
3. MTGase exhibited the strengthening effect on surimi gels. The use of microbial transglutaminase in combination with fish gelatin at appropriate level could modify properties of surimi gel, especially via improving water holding capacity of gel.
4. The mixtures of gelatins from different sources could be used to improve the properties of gelatin gels. Nevertheless, the ratio of various gelatins should be optimized.
5. Gelatin from skin of unicorn leatherjacket could be used as non-muscle collagenous protein additive. However, MTGase should be used to maintain or improve mechanical properties of resulting gels.

6.2 Future works

1. Modification of gelatin should be carried out to improve their interaction with myofibrillar protein in surimi.

2. Effects of gelatin from different sources with various molecular properties on surimi gel characteristic should be investigated.

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Kaewudom, P., Benjakul, S. and Kijroongrojana, K. 2011. Effect of bovine and fish gelatin in combination with microbial transglutaminase on gel properties of threadfin bream surimi. Int. Aquat Res. (In revision).

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