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Effect of bovine and fish gelatin in combination with microbial transglutaminase on gel properties of threadfin bream surimi

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Abstract

Textural property of surimi products is a prime factor in determining the acceptability of consumer as well as market value. Gelatin is one of the most popular biopolymers widely used in food industry as gelling agent with the unique textural properties. Therefore, the addition of 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. Surimi from the threadfin bream (*Nemipterus bleekeri*) was 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 microbial transglutaminase (MTGase) at 1.2 units/g surimi. Textural properties, whiteness, expressible moisture content, protein pattern, and microstructure and sensory properties 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 gels, with and without 10% BG added ($p < 0.05$). On the other hand, 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 lowered the expressible moisture content and whiteness of surimi gel ($p < 0.05$). Based on SDS-PAGE, band intensity of myosin heavy chain 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. Gelatin, especially bovine/fish gelatin mix, at an appropriate level could be used as the protein additive in surimi gel in conjunction with MTGase in order to improve the textural and nutritive properties of the products.

Keywords: Surimi, Gel fish, Gelatin, Bovine gelatin, Microbial transglutaminase, Cross-linkers

Background

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 (FG) was added into surimi gel, but it resulted in the decrease in gel strength (Hernandez-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 byproducts 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 metric tons, respectively (Pangsorn et al. 2007). Surimi can be used to prepare a variety of processed foods such as kamaboko, kani (crab) -kamaboko, chikuwa, Satsuma-age, 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 or protein cross-linkers such as microbial transglutaminase (MTGase) have been widely used in surimi (Benjakul and Visessanguan 2003; Benjakul et al. 2004a). MTGase from *Streptovorticillium mobaraense* does not require calcium ions for its 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). The 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 the textural, physical, and sensory properties of surimi gel from threadfin bream surimi added with bovine and fish gelatin mix in combination with MTGase.

Methods

Chemicals/gelatin

Sodium dodecyl sulfate (SDS), β -mercaptoethanol (β ME), glycerol, high molecular weight marker, and glutaraldehyde were purchased from Sigma (St. Louis, MO, USA). *N, N, N', N'*-tetramethyl ethylene diamine and all chemicals for electrophoresis were procured from Bio-Rad Laboratories (Hercules, CA, USA). Bovine hide gelatin and fish gelatin were purchased from Halamic Company (Bangkok, Thailand) and LAPI GELATINE S.p.A. (Empoli, Italy), respectively. MTGase from *S. mobaraense* was supplied by Ajinomoto 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.

Preparation of surimi gel added with gelatins 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 to 10 h to obtain the core temperature of approximately 0°C to 2°C. Surimi was then cut into small pieces and mixed with 2.5% NaCl in a mixer (MK-5087 M, 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 mixes (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 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 the 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.

Determination of properties of surimi gel

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, Surrey, UK) following the method of Benjakul et al. (2007). Gels were equilibrated at room temperature (28°C to 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.

Texture profile analysis

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

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 to 4 g), weighed accurately (X), and placed between three pieces of Whatman paper No.1 at the bottom and two pieces on the top of the sample. The standard weight (5 kg) was placed on the top and held for 2 min. The samples were then removed from the papers and weighed again (Y). Expressible moisture content was calculated with the following equation:

$$\text{Expressible moisture content (\%)} = \left[\left(X - \frac{Y}{X} \right) \right] \times 100.$$

Whiteness

Whiteness of gel samples was determined as described by Benjakul et al. (2004b) 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^* is lightness; a^* is redness/greenness; and b^* is yellowness/blueness.

SDS-polyacrylamide gel electrophoresis

Protein patterns of gels were analyzed under reducing condition by sodium dodecyl sulfate polyacrylamide gel electrophoresis (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 was 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 $3,500 \times g$ 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 ME, 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 μ g 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.

Microstructure of surimi gel

The 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 a scanning electron microscope. Gel samples were cut into small pieces ($0.25 \times 0.25 \times 0.25 \text{ cm}^3$) and fixed with 2.5% glutaraldehyde in 0.2 M phosphate buffer, in 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).

Sensory evaluation

Surimi gels 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.

Statistical analysis

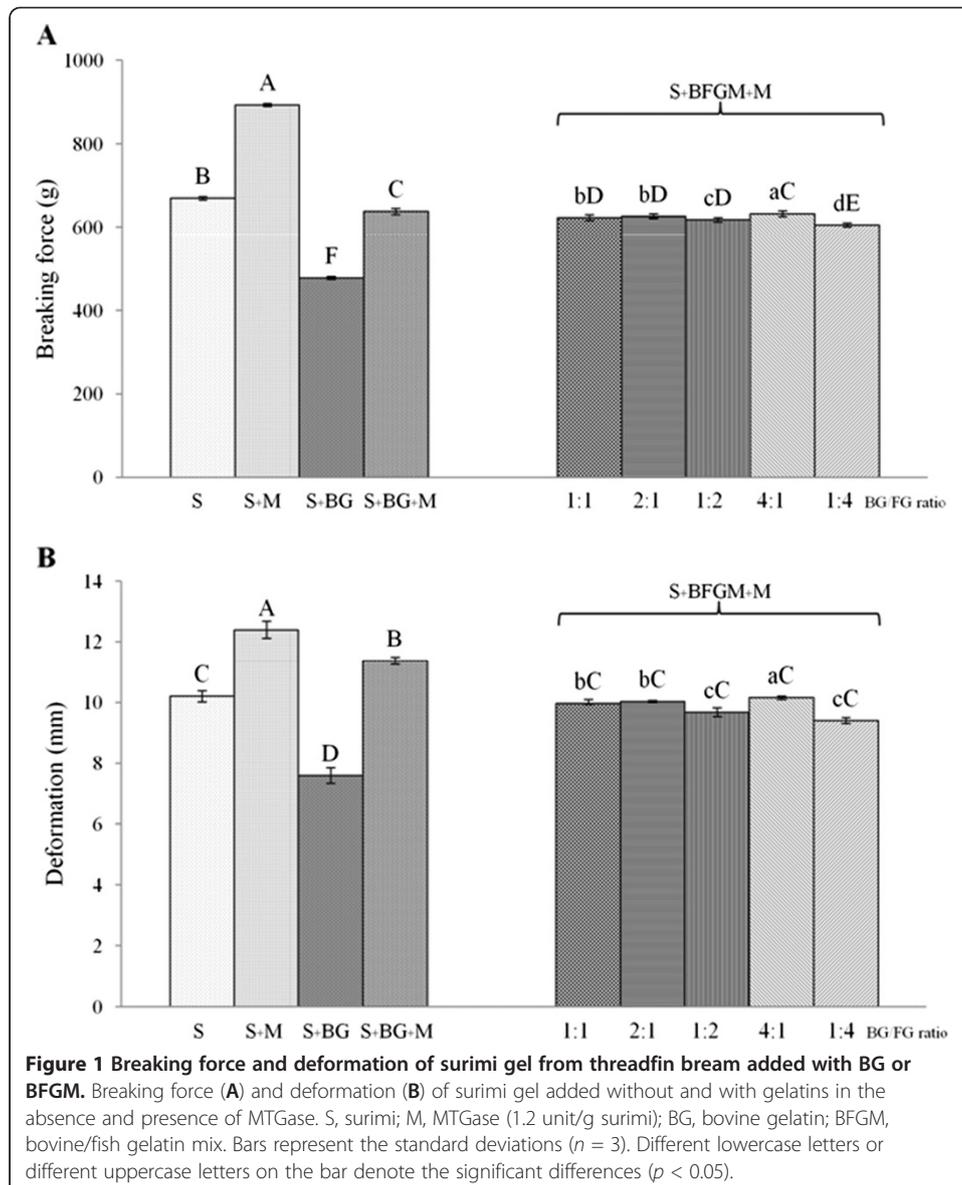
All experiments were ran in triplicate. Completely randomized design was used for the entire study. Data were subjected to analysis of variance, and the mean comparisons were carried out using Duncan's multiple range test (Steel and Torrie 1980). Statistical analysis was performed using the Statistical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

Results and discussion

Breaking force and deformation of surimi gel added with gelatins in combination without and with 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 1. 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 (Hernandez-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), alginate, 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 Hernandez-Briones et al. (2009) who reported that surimi gel from Alaska pollock had the decreases in shear stress and shear strain 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 the breaking force and deformation of resulting gels by 33.4% to 49.7%, compared with that containing 10% BG. MTGase is an enzyme that catalyzes the cross-linking of proteins through the formation of covalent bonds between protein molecules. The addition of MTGase induced the cross-linking of myosin heavy chain (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 with 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 with 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 possibly due to the lower gel forming ability of the former. Also, FG might be less cross-linked by MTGase, compared with BG. It was reported that mammalian gelatin had a higher content of lysine, compared with fish gelatin (Haug et al. 2004;



Schrieber and Gareis 2007). Lysine has been known to serve as an acyl acceptor for protein cross-linking induced by MTGase (Seguro et al. 2006). As a result, the poorer gel with lower cross-links was attained when FG was present.

Gel strength is the major physical property of gelatin gels. This is 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 $100,000 \text{ g mol}^{-1}$. 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, such as 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). Furthermore, the higher FG ratio in BFGM resulted in the higher decrease in deformation of surimi gel.

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

Expressible moisture content of surimi gel added with BG and BFGM in the presence and absence of MTGase is shown in Table 1. 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. Hernandez-Briones et al. (2009) reported that the surimi gels from Alaska pollock surimi containing 7.5 to 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 ($p < 0.05$). The 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.

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

The decreases in whiteness were found in surimi gels added with BG or all BFGM ($p < 0.05$) (Table 1). 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

Table 1 Expressible moisture content and whiteness of surimi gel from threadfin bream added with BG or BFGM

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

Surimi gels containing 10% BF or 10% BFGM without and with 1.2 units/g MTGase. ^aValues are mean ± SD ($n = 3$). Different lowercase superscripts (b and c) in the same column denote the significant differences ($p < 0.05$). BF, bovine gelatin; BFGM, bovine/fish gelatin mix; MTGase, microbial transglutaminase.

samples tested ($p > 0.05$). The 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 et al. 2007; Benjakul and Visessanguan 2000; Benjakul et al. 2001).

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

Protein patterns of surimi gels added without and with gelatin in the presence or absence of MTGase are depicted in Figure 2. 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). 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) were added, compared with that observed in the control gel (without 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, there is no difference in MHC band intensity of surimi gel added with BFGM having different ratios of BG and FG. The result suggested that MHC underwent polymerization at similar extent, regardless of gelatin addition and types of gelatin.

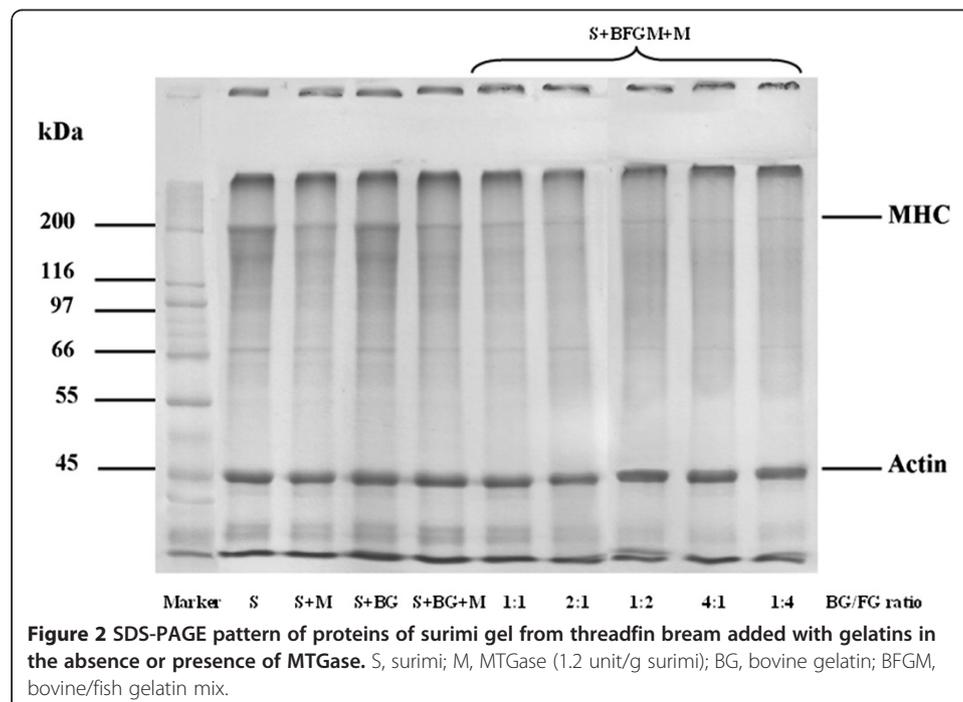


Figure 2 SDS-PAGE pattern of proteins of surimi gel from threadfin bream added with gelatins in the absence or presence of MTGase. S, surimi; M, MTGase (1.2 unit/g surimi); BG, bovine gelatin; BFGM, bovine/fish gelatin mix.

Table 2 Textural properties of surimi gel from threadfin bream containing 10% BF or 10% BFGM without and with 1.2 units/g MTGase

Samples	Hardness (N) ^a	Springiness (cm) ^a	Cohesiveness (ratio) ^a	Gumminess (N) ^a	Chewiness (N cm) ^a
Surimi (without gelatin and MTGase)	89.51 ± 0.16 ^c	0.91 ± 0.12 ^c	0.53 ± 0.01 ^b	49.16 ± 0.13 ^c	46.37 ± 0.15 ^b
Surimi + MTGase (without gelatin)	98.97 ± 0.13 ^b	0.98 ± 0.37 ^b	0.60 ± 0.00 ^b	57.49 ± 0.14 ^b	49.67 ± 0.22 ^b
Surimi + 10% BG (without MTGase)	72.11 ± 0.61 ^f	0.71 ± 0.00 ^e	0.45 ± 0.00 ^d	39.91 ± 0.72 ^e	36.19 ± 0.36 ^c
Surimi + 10% BG with MTGase	88.86 ± 0.42 ^c	0.91 ± 0.01 ^c	0.56 ± 0.00 ^b	47.90 ± 0.22 ^c	45.79 ± 0.11 ^b
Surimi + 10% BFGM (the mixture of BG:FG, 1:1) with MTGase	86.69 ± 0.78 ^c	0.86 ± 0.00 ^d	0.51 ± 0.05 ^c	43.81 ± 0.18 ^d	40.18 ± 0.15 ^c
Surimi + 10% BFGM (the mixture of BG:FG, 2:1) with MTGase	87.03 ± 0.61 ^c	0.88 ± 0.00 ^d	0.51 ± 0.51 ^c	45.00 ± 0.72 ^c	44.19 ± 0.36 ^b
Surimi + 10% BFGM (the mixture of BG:FG, 1:2) with MTGase	83.90 ± 0.42 ^d	0.86 ± 0.01 ^d	0.50 ± 0.02 ^c	42.90 ± 0.22 ^d	39.79 ± 0.11 ^c
Surimi + 10% BFGM (the mixture of BG:FG, 4:1) with MTGase	87.42 ± 0.47 ^c	0.88 ± 0.01 ^d	0.52 ± 0.00 ^{bc}	47.01 ± 0.50 ^c	45.07 ± 0.58 ^b
Surimi + 10% BFGM (the mixture of BG:FG, 1:4) with MTGase	80.37 ± 0.78 ^e	0.85 ± 0.00 ^d	0.49 ± 0.05 ^c	42.81 ± 0.18 ^d	37.18 ± 0.15 ^c

^aValues are mean ± SD (n = 3). Different lowercase superscripts (b to f) in the same column denote the significant differences (p < 0.05). BF, bovine gelatin; BFGM, bovine/fish gelatin mix; MTGase, microbial transglutaminase.

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

TPA parameters of surimi gels added without and with 10% BG or BFGM in the presence and absence of MTGase are depicted in Table 2. 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 1). 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 the 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 FS/BS ratio increased. However, the highest chewiness was obtained when BFGM with FS/BS 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.

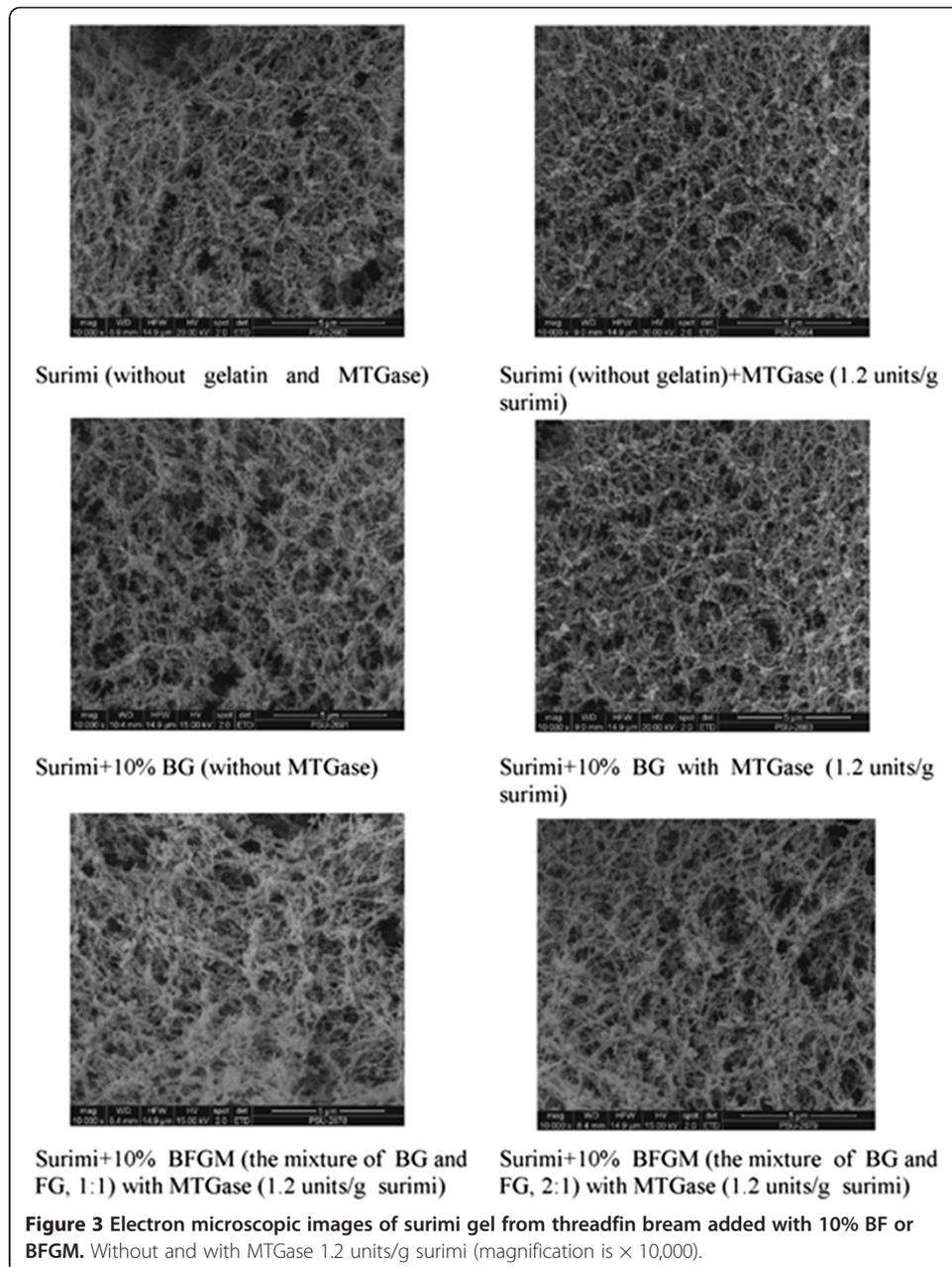
Likeness score of surimi gel added with gelatins in combination without and with MTGase

Likeness score of surimi gels added without and with gelatin in the presence and absence of MTGase is shown in Table 3. There was a 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 1). The excessive

Table 3 Likeness score of surimi gel from threadfin bream containing 10% BF or 10% BFGM without and with 1.2 units/g MTGase

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

^aValues are mean ± SD ($n = 3$). Different lowercase superscripts (b and c) in the same column denote the significant differences ($p < 0.05$). BF, bovine gelatin; BFGM, bovine/fish gelatin mix; MTGase, microbial transglutaminase.



formation of ϵ -(γ -glutamyl) lysine cross-links would yield the gel with rigid and tough texture. There is 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 FS/BS 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 an interfering effect of fish skin gelatin on surimi gel property as well as sensory property. However, gel added with MTGase and BFGM, having FS/BS 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 FS/BS ratio could be added into surimi along with MTGase to yield the gel with acceptability.

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

Microstructures of surimi gels added without and with MTGase in the presence of 10% BG or BFGM at different BG/FG ratios are illustrated in Figure 3. 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 (Benjakul et al. 2008). As a consequence, a more compact and denser gel network was developed. The result was in accordance with the higher breaking force and deformation (Figure 1), and the lowered expressible moisture content (Table 1) when MTGase was added in surimi. The coarser structure with a larger void was obtained when 10% BF 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.

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.

Abbreviations

β ME: β -mercaptoethanol; BFGM: bovine/fish gelatin mix; BG: bovine gelatin; FG: fish gelatin; MHC: myosin heavy chain; MTGase: microbial transglutaminase; SDS: sodium dodecyl sulfate; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; SPSS: Statistical Package for Social Science.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SB formulated the hypothesis and designed the studies. PK conducted the experiments and analyses. KK was involved in sensory evaluation. PK and SB wrote the paper. All authors read and approved the final manuscript.

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