

# Thermal and physicochemical properties of red tilapia (*Oreochromis niloticus*) surimi gel as affected by microbial transglutaminase

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**Abstract.** Thermal and physicochemical properties of red tilapia (*Oreochromis niloticus*) surimi gel incorporated with different levels of microbial transglutaminase (MTGase) were investigated. Surimi samples mixed with various concentrations of MTGase were subjected to two-stages heating (at 45°C followed by 90°C) to prepare surimi gel. Samples formulated with 0.30 MTGase (units/g surimi) showed the highest breaking force and deformation, and lowest expressible water content among treatments. Highest storage modulus was found in the gels mixed with 0.30 MTGase (units/g surimi). Compared with control surimi gel, addition of microbial transglutaminase to levels 0.10, 0.20 and 0.30 (units/g surimi) increased the enthalpy and maximum transition temperature of myosin. Results suggest that up to 0.30 MTGase (units/g surimi) could improve texture, colour, water-holding capacity, elasticity and thermal stability of red tilapia surimi gel.

**Additional keywords:** differential scanning calorimetry, expressible water, storage modulus, textural properties, whiteness.

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## Introduction

Surimi is a pure protein paste made of minced and washed fish meat stabilised by cryoprotectants (Park and Lin 2005). Surimi products were first produced in Japan, but they are now known in many parts of the world through products such as fish balls, fish cake and crab meat analogues. Gel properties of surimi such as texture and gel-forming (gelation) ability are the major factors determining quality of surimi and related products (Yin and Park 2014). Gelation of fish proteins is the most important step in forming desired textures in surimi-based products. Depending on fish species, type of applied additives and preparation method, surimi gels with different properties and stabilities can be formed (Yongsawatdigul *et al.* 2002; Benjakul and Visessanguan 2003; Ramirez *et al.* 2007; Kaewudom *et al.* 2012).

Gelation process is associated with cross-linking of myosin heavy chains (MHC), induced by endogenous transglutaminase (TGase). Endogenous TGase is an enzyme catalysing covalent bonding between the  $\epsilon$ -amino group of lysyl residues and the  $\gamma$ -carboxamide group of glutamyl residues of adjacent protein molecules (Yongsawatdigul *et al.* 2002). Reactions catalysed by TGase result in significant changes in the physicochemical properties of gel such as changes in viscosity, thermal stability

and elasticity (Kuraishi *et al.* 1997; Yokoyama *et al.* 2004). Endogenous TGase is a water-soluble enzyme and can be partially removed during washing step in surimi preparation process. Yongsawatdigul *et al.* (2002) investigated the residual TGase activity in threadfin bream surimi wash water, confirming that ~44% of original TGase activity remained in the final surimi after the washing process.

Thermal and physicochemical properties of surimi gel can be improved by the addition of food-grade ingredients and additives such as egg white (Yetim and Ockerman 1995), casein and beef plasma-thrombin (Baker *et al.* 2000) and whey protein concentrate (Rawdkuen *et al.* 2008) during surimi and surimi gel preparation. Dondero *et al.* (2006) reported that addition of 0.10% to 0.50% (surimi weight basis) of microbial TGase (MTGase) improved the textural quality of surimi gels produced from Chilean jack mackerel (*Trachurus murphyi*) by enhancement of  $\epsilon$ -( $\gamma$ -glutamyl) lysine bonds production.

MTGase has been widely used to improve protein gel texture including surimi gel (Cardoso *et al.* 2010; Hemung and Chin 2013). MTGase is a calcium independent enzyme that can be derived from microorganisms such as *Streptoverticillium mobaraense* and *Streptomyces lydicus* and can be used as a

food-grade additive. Data from several sources indicate that the efficiency of MTGase-induced improvement in the properties of gel depends on fish species and the amount of MTGase added. A research done on surimi from lizardfish (*Suarida tumbil*) incorporated with MTGase revealed that the addition of MTGase (0.40 units/g surimi) was able to produce stronger gel (Chanarat *et al.* 2012), whereas the optimal amount of MTGase was 0.30 units/g for threadfin-bream (*Nemipterus bleekeri*) surimi, and 0.20 units/g surimi for pollack (*Theragra chalcogramma*) (Jiang *et al.* 2000). Red tilapia is one of the main economic freshwater fish species in Malaysia, but consumption of red tilapia is limited due to its muddy and fishy odour and its large number of intramuscular small bones (Rawdkuen *et al.* 2009). However, surimi and surimi products is a plausible choice for processing red tilapia meat. In the case of red tilapia surimi, there is insufficient information about the impact of different concentrations of MTGase on the surimi gel properties. Hence, this study aimed to investigate the effect of different levels of MTGase and determine the appropriate concentration of MTGase for improving the quality of red tilapia surimi gel.

## Material and methods

### Raw material and additive

Red tilapia fish were purchased from the wholesale fish market, Selangor, Malaysia. The fish was kept on ice at a fish : ice ratio of 1 : 2 (w/w) and transported within 2 h to the Fish Laboratory of the Department of Food Technology, Universiti Putra Malaysia. The fish was gutted, headed and washed to use for surimi and surimi gel preparation.

MTGase from *Streptovorticillium mobaraense* (TG-K) with activity of 100 units/g dry matter was supplied by Ajinomoto, Malaysia.

### Surimi preparation

The surimi preparation procedure was performed according to the method of Benjakul *et al.* (2005). The washed and gutted fish was mechanically deboned using a deboning machine (Fish meat separator, Model FD 6, Kedah, Malaysia) to obtain minced fish meat. The mince was washed with cold water (5°C) in the holding tank at a mince: water ratio of 1 : 3 (w/v). The mixture was manually stirred for 5 min and the water was removed by tilting the tank and squeezing manually. The washing and dewatering process were repeated three times. After the third washing, the mince was filtered using a nylon screen. The mince was mixed with 4% sucrose and 4% sorbitol in a domestic blender (KitchenAid, Model 5K5SS, Greenville, OH, USA) for 5 min and then packed into a 1-kg polyethylene bag and kept at -18°C for further treatment and analysis.

### Surimi gel processing and treatments

Frozen surimi was thawed with running water (25°C) until the temperature of the surimi block reached 5°C. After thawing, surimi was cut into small pieces (~1 cm cube), blended with 2.5% sodium chloride, and mixed with crushed ice to adjust surimi moisture content to 80% [monitored using moisture analyser (Precisa Gravimetrics AG, CH-8953, Dietikon, Switzerland)]. MTGase at different levels (0.10, 0.20, 0.30,

0.40 and 0.50 units/g surimi) was added and the mixture was homogenised in a domestic blender (Pensonic, Model PB-326, Johor Bahru, Malaysia) at a speed of 7000g at 5°C for 5 min. Surimi with no additive was used as the control. The obtained paste (500 g) was placed in a cylindrical polyvinylidene chloride casing (3 cm diameter and 15 cm height) and sealed tightly at both ends. The surimi gel was prepared by using two-stage heat treatment in a water bath, first at 45°C for 30 min and then at 90°C for 20 min. The heated surimi gel was cooled at room temperature and kept for 24 h at 4°C before analysis.

### Texture analyses

Determination of the breaking force (gel strength), and deformation (elasticity/deformability) of surimi gels were accomplished according to the method described by Zhou *et al.* (2006) using a texture analyser TA.XT2i (Stable Micro Systems, Surrey, UK). The cylinder-shaped samples were cut to a height of 2.5 cm and subjected to a double compression test by a spherical plunger (5 mm diameter) to 75% of the original height at a speed of 60 mm/min. The breaking force [the force (g) to puncture into the gel] and deformation [the distance (mm) at which the probe punctured into the gel] were measured and reported.

### Expressible water content

The expressible water (EW) for each treatment was measured according to the method of Mao and Wu (2007). Approximately 3 ± 0.20 g of samples were weighed and put between two filter papers (Whatman No.1). Samples were placed at the bottom of 50-mL centrifuge tubes and centrifuged at 5000g for 20 min at 15°C. Immediately after centrifugation, the samples were weighed and the EW was calculated as follows:

$$EW (\%) = [(W_i - W_f) / W_i] \times 100,$$

where  $W_i$  was the initial weight of surimi gel and  $W_f$  was the final weight of surimi gel.

### Whiteness

The reflected colour of the surimi gels from red tilapia surimi formulated with different levels of MTGase was determined using a Minolta Croma Meter Model CR-300 (Minolta Co., Tokyo, Japan). The colourimeter was standardised with a standard-white reflection plate using a standard 3–4 white tile ( $L^*$  value of 96.35,  $a^*$  value of 0.28 and  $b^*$  value of 1.68). Before measuring, the surface of samples was lightly dried with paper towels.  $L^*$  (lightness),  $a^*$  (redness/greenness) and  $b^*$  (yellowness/blueness) were measured in five replications for each sample and whiteness was calculated as described by Shie and Park (1999) as follows:

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

### Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

Protein patterns of all treatments were analysed on SDS-PAGE according to the method of Laemmli (1970). Protein sample was prepared according to the method of Benjakul *et al.* (2006).

To prepare the sample, 27 mL of 5% SDS solution was heated to 85°C for 1 h. The heated solutions were added to 3 g of the sample and homogenised for 2 min using a domestic homogeniser (Pensonic, Model PB-326) at a speed of 7000 rpm. The mixture was then incubated at 85°C for 1 h to dissolve proteins. The samples were centrifuged at 5000g at 5°C for 15 min to remove undissolved residues. Protein concentration in the supernatant was determined as per the method of Lowry *et al.* (1951). Samples (15 µg protein) were loaded on the polyacrylamide gel (12.50% separation gel and 4% stacking gel). Electrophoresis was carried out at 20-mA constant current in 1xTAE (TRIS-acetate-EDTA) buffer using a Mini Protein II unit (BioRad Laboratories, Berkeley, CA, USA). Subsequently, the polyacrylamide gels were stained with Coomassie brilliant blue R-250 overnight and then destained in a solution containing 15% methanol (v/v) and 10% (v/v) acetic acid. A protein standard (Bio-Rad Laboratories) ranged from 10 to 250 kDa was used as the marker.

#### Dynamic test

The storage (elastic) modulus ( $G'$ ) of the surimi from red tilapia mixed with different levels of MTGase in a course of heating from 20°C to 90°C were measured using a Thermo Electro Corporation Rheostress (Haake, RT 20, Rotovisco, Berlin, Germany) according to the method of Rawdkuen *et al.* (2008). The Rheostress was equipped with C35/2°Ti cone and plate geometry. An oscillation of 0.1 Hz with a resistance stress of 3 Pa was used for the testing. A plastic cover supplied by the manufacturer was used to prevent moisture evaporation during analysis.

#### Differential scanning calorimetry

Thermal properties of surimi gels were studied using a differential scanning calorimetry (DSC7, Perkin-Elmer, Norwalk, CT, USA) by monitoring of the maximum temperature of denaturation and enthalpy, according to the method of Karayannakidis *et al.* (2008) with slight modification. Samples (surimi paste mixed with different levels of MTGase) of 10 ± 0.01 mg were weighted and placed in a 50-µL Perkin-Elmer aluminium pans, then capped and sealed. The prepared sample was scanned from 27°C to 100°C at a heating rate of 5°C/min. An empty pan was used as a reference. Maximum temperature of denaturation and changes in enthalpy were measured from the trace.

#### Statistical design and analyses

Completely randomised design was employed for statistical design of this study. All data were analysed by one-way ANOVA using a Minitab software (Version 16.0 software, Minitab Inc., State College, PA, USA) to determine the effect of MTGase at different levels on the surimi gel. Tukey's test was used to carry out the difference of means between pairs with 95% confidence interval.

## Results and discussion

#### Textural properties

Breaking force and deformation of surimi gels containing various levels of MTGase are shown in Table 1. Different

**Table 1. Breaking force and deformation of red tilapia surimi gels affected by different concentrations of microbial transglutaminase (MTGase)**

The numbers represent mean ± s.d. of three replications. Different letters in the same column indicate the significant differences ( $P < 0.05$ )

Additive	Level (units/g surimi)	Breaking force (g)	Deformation (mm)
Control	0.00	434.36 ± 2.60a	10.50 ± 0.08a
TGase	0.10	435.17 ± 3.07a	10.60 ± 0.10a
	0.20	438.00 ± 2.80a	13.13 ± 0.60b
	0.30	763.70 ± 10.76d	14.79 ± 0.40d
	0.40	528.66 ± 4.40c	14.31 ± 0.30c
	0.50	460.85 ± 2.46b	14.05 ± 0.50c

concentrations of MTGase generally had significant effect ( $P < 0.05$ ) on breaking force and deformation. MTGase addition at the level of 0.1 and 0.2 units/g surimi did not significantly affect the breaking force and deformation in comparison with control gel. The breaking force and deformation significantly increased with the addition of MTGase at 0.3 units/g surimi and decreased significantly with the addition of more than 0.3 MTGase (units/g surimi). Among all samples mixed with MTGase, those containing 0.30 MTGase (units/g surimi) showed the highest breaking force (763.70 ± 10.76) and deformation (14.79 ± 0.40), whereas those added with 0.10 MTGase (units/g surimi) had the lowest breaking force (435.17 ± 2.60) and deformation (10.60 ± 0.08). Several reports have shown that the use of MTGase above an optimum concentration caused a detrimental effect on the textural properties of surimi gel (Lee *et al.* 1997; Ramirez *et al.* 2000). These results are in accord with those of obtained by Jiang *et al.* (2000) who reported that breaking force and deformation of surimi gels from threadfin-bream (*Nemipterus virgatus*) and pollock (*Rastrelliger kanagurta*) increased when MTGase increased up to a certain level (0.30 unit MTGase/g surimi) and further increase in MTGase decreased the gel strength of surimi gels. Dondero *et al.* (2006) reported maximum of 364% increase in gel strength in surimi made from jack mackerel (*T. murphyi*) when 0.20% TGase applied. The addition of MTGase at up to 2 g/kg to red tilapia surimi showed the highest breaking force, which was increased by 240% compared with surimi with no additive (Duangmal and Taluengphol 2010). MTGase induces the formation of non-disulfide covalent bonds (Tammattinna *et al.* 2007), which results in the formation of MHC cross-linking and subsequently, a strong gel (Chaijan and Panpipat 2010). Addition of TGase to a gelatin from megrim (*Lepidorhombus boscii*) skins beyond a certain concentration (0.30%) resulted in a decrease in the gel strength (Jongjareonrak *et al.* 2006). Decrease in breaking force and deformation of surimi gel with more than 0.30 MTGase (units/g surimi) might be due to excessive cross-linking, which lower the gel strength through impeding intermolecular aggregation that reduced the gel network formation (Jongjareonrak *et al.* 2006).

#### Expressible water content

Content of EW is associated with the water-holding capacity of the surimi gel. There is a close relationship between gel texture

and water-holding capacity and subsequently gel textural properties of cooked gel (Lee 1984). Expressible moisture content of surimi gels from red tilapia treated with MTGase at different levels is shown in Table 2. EW value of control surimi gel was  $11.70 \pm 0.05\%$  whereas the lowest ( $10.50 \pm 0.02\%$ ) EW was found in surimi gels containing 0.30 MTGase (units/g surimi). Surimi gels showed a continuous decrease in EW content when the levels of MTGase increased up to 0.30 units/g surimi ( $P < 0.05$ ). However, increase in EW content was noticeable when higher levels of MTGase were added ( $P < 0.05$ ). A lower EW indicates a higher water-holding capacity of the gel matrix (Ramírez *et al.* 2000; Tammattinna *et al.* 2007). MTGase-induced protein cross-linking may result in more water being retained inside the gel matrix. Chaijan and Panpipat (2010) investigated the effect of TGase on gel-forming ability of mackerel (*Rastrelliger branchysoma*) protein and indicated that water-holding capacity of surimi gels increased when the concentration of TGase increased up to 0.25 (unit/g of surimi). The addition of MTGase to red tilapia surimi gel led to an increase in the observed EW compared with the control, especially with 3 g/kg MTGase additive (Duangmal and Taluengphol 2010). The stronger gel network was possibly linked with its capacity to hold water. However, TGase can also increase protein–protein interactions, leading to a decrease in protein–water interaction. Therefore, water-holding capacity of gels can decrease when high concentrations of MTGase are used (Kaewudom *et al.* 2013).

### Whiteness

Colour is an important indicator in surimi gel quality and generally, high whiteness commands better value back as a result of higher demand. Different levels of MTGase had significant ( $P < 0.05$ ) effect on red tilapia surimi gel whiteness compared with the control (Table 2). Whiteness of surimi gels increased significantly ( $P < 0.05$ ) when MTGase concentration increased. However, there were slight differences in the whiteness of surimi gels mixed with 0.30–0.50 MTGase (units/g surimi). Surimi gels with 0.50 MTGase (units/g surimi) showed the highest value ( $80.20 \pm 0.12$ ) of whiteness compare to that of the control ( $76.53 \pm 0.20$ ).

This result was in accordance with Karayannakidis *et al.* (2008) who reported that the addition of MTGase had beneficial effect on whiteness of surimi gels from sardine (*Sardinella*

*gibbosa*). Duangmal and Taluengphol (2010) also showed that MTGase at levels of 1, 2 and 3 g/kg increased whiteness of surimi. As transglutaminase catalyses the cross-linking reaction of myosin and leads to the formation of protein intra- and intermolecular covalent bonds (Lee 1984), it was concluded that differences in the whiteness value could correspond to the increased turbidity of gels as a result of TGase activity. Moreover, increase in whiteness could be caused by other compounds (lactose, maltodextrin) in commercial MTGase powder, which cause light scattering effect (Chanarat *et al.* 2012).

### Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

The protein profiles of surimi gels incorporated with MTGase at various concentrations are shown in Fig. 1. Surimi gel contained MHC and actin as the major proteins. Two bands ~43 kDa and 205 kDa range were those of actin and MHC, respectively (Thorarinsdottir *et al.* 2002).

The visual presence of MHC band intensity decreased as the amount of MTGase increased. The MHC distinct band nearly disappeared with 0.30 MTGase (units/g surimi). This might be associated with formation of non-disulfide covalent cross-linking, which is contributed partially to gel strength enhancement (Ko *et al.* 2007; Yin and Park 2014). These findings are in agreement with the findings of Dondero (2006), who reported that the content of MHC decreased and the cross-linked protein amount increased with increasing setting time and addition of TGase in Chilean jack mackerel (*Trachurus murphyi*) surimi. SDS-PAGE pattern from surimi gels of threadfin-bream (*Nemipterus virgatus*) and pollack (*Rastrelliger kanagurta*) surimi with TGase showed that inter- and/or intra-molecular cross-linking formed in MHC band cross-linked during heating (Benjakul *et al.* 2006; Chanarat *et al.* 2012). Moreover, study conducted by Duangmal and Taluengphol (2010) on surimi showed MTGase addition decreased the intensity of the MHC band significantly. The decrease in MHC band could be due to of protein bonds formation of the  $\epsilon$ -( $\gamma$ -glutamyl) lysineiso in cross-linked MHC in the presence of MTGase. Polymerised myosin could not go into the SDS gel, leading to the decline of the band intensity (Benjakul *et al.* 2002). There is no marked difference in actin band intensity of surimi gels formulated with different levels of MTGase. It can be suggested that actin was not a preferred substrate of TGase or actin affected gel functionality to a lesser extent (Yin and Park 2014).

### Storage modulus

Storage modulus (elastic modulus) has been used to study the heat-induced gelation of myofibrillar proteins. Changes in maximum storage modulus ( $G'_{\max}$ ) of red tilapia surimi gels affected by different concentrations of MTGase are shown in Table 3. Three obvious peaks of storage modulus were observed during heating. The first peak of  $G'$  at ~40°C may have resulted from the tail portion of the myosin molecule via hydrophobic interactions, bringing the cross-linking between the protein molecules. This is believed to be associated with the

**Table 2. Whiteness and expressible water values of red tilapia surimi gels affected by different concentrations of microbial transglutaminase (MTGase)**

The numbers represent mean  $\pm$  s.d. of three replications. Different letters in the same column indicate the significant differences ( $P < 0.05$ )

Additive	Level (units/g surimi)	Whiteness	Expressible water (%)
Control	0.00	$76.53 \pm 0.20$ a	$11.70 \pm 0.05$ d
TGase	0.10	$78.38 \pm 0.16$ b	$11.25 \pm 0.03$ b
	0.20	$78.02 \pm 0.14$ b	$11.13 \pm 0.04$ b
	0.30	$79.73 \pm 0.26$ c	$10.50 \pm 0.02$ a
	0.40	$79.98 \pm 0.18$ c	$11.56 \pm 0.08$ c
	0.50	$80.20 \pm 0.12$ c	$11.75 \pm 0.01$ c

high-temperature setting called suwari phenomenon (Chen *et al.* 2005).

The second peak was observed  $\sim 68^\circ\text{C}$ . This step was referred as gel strengthening and was accompanied by an increase in the number of cross-links between protein aggregates and a deposition of additional denatured proteins in the existing protein network to strengthen the gel matrix (Chen *et al.* 2005). Xiong (1997) also reported that the increase in  $G'$  up to  $70^\circ\text{C}$  indicates the formation of a highly elastic myofibrillar protein gel in modori stage. By increasing the temperature over  $76^\circ\text{C}$  (third peak), transformation of the samples to final stage called kamaboko phenomenon happened which was associated with a sharp increase in  $G'$  values (Benjakul *et al.* 2002).

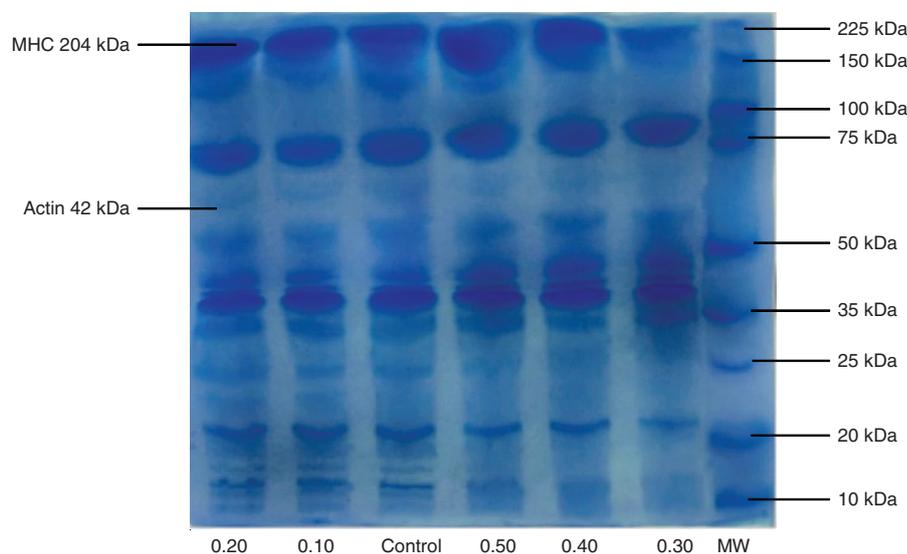
Values of  $G'$  of the peaks ( $G'_{\max}$ ) were significantly ( $P < 0.05$ ) different in all samples. The highest  $G'$  for the three peaks was observed in the surimi gels mixed with 0.30 MTGase (units/g surimi), which were  $2688 \pm 11.00$  Pa at  $42.10^\circ\text{C}$ ,  $4792 \pm 6.00$  at  $68.80^\circ\text{C}$  and  $5878 \pm 9.00$  Pa at  $77.50^\circ\text{C}$ . Greater  $G'_{\max}$  values indicated higher elasticity of formulated surimi gels. This behaviour could be due to slower protein aggregation than denaturation, resulting in higher elasticity in gels during

heating (Xiong 1997). Higher  $G'_{\max}$  could also be due to more cross-linking of proteins, which also increases the amount of energy stored in the gel matrix (Kyaw *et al.* 2001).

Decrease in maximum storage modulus in surimi gels with higher levels MTGase (0.40 and 0.50 units/g surimi) might be due to the increase in formation of transglutaminase-mediated covalent bonds, thus delaying the intermolecular aggregation leading to reduced elasticity of the gel (Yongsawatdigul and Piyadhamviboon 2005).

#### Differential scanning calorimetry

Enthalpy and maximum temperature of denaturation are two main factors in determination of thermal properties of fish and fish products, which can be measured by DSC. Maximum temperature of denaturation ( $T_m$ ) is protein's heat uptake that is required to denature protein structure (Norziah *et al.* 2009). The enthalpy value is evaluated from the space under the transition peak, which is associated with the secondary structure of protein (Spink 2008). Maximum temperature of denaturation and enthalpy of peaks from the control and samples mixed with MTGase at various levels were recorded by DSC and



**Fig. 1.** SDS-PAGE pattern of red tilapia kamaboko gels affected by different concentrations of microbial transglutaminase (MTGase; units MTGase/g surimi).

**Table 3.**  $G'_{\max}$  (Pa) and  $T_m$  ( $^\circ\text{C}$ ) of three clear peaks observed in the rheometer measurement of red tilapia surimi gels treated with different concentrations of microbial transglutaminase (MTGase)

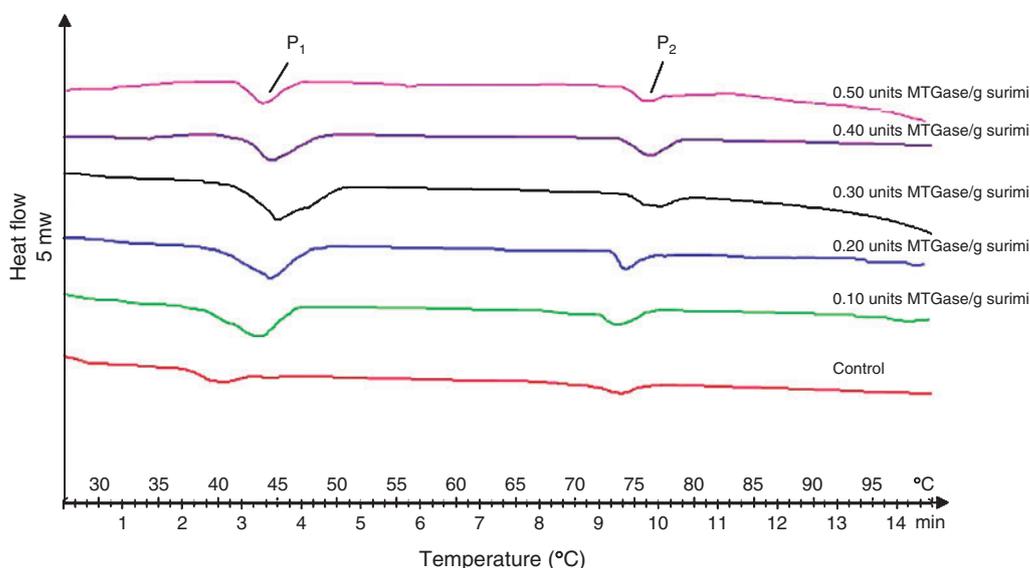
The numbers represent mean  $\pm$  s.d. of three replications. Different letters in the same column indicate significant differences ( $P < 0.05$ )

Additive	Level (units/g surimi)	Peak 1		Peak 2		Peak 3	
		$G'_{\max}$	$T_m$	$G'_{\max}$	$T_m$	$G'_{\max}$	$T_m$
Control	0.00	$2232 \pm 6.00a$	$41.20 \pm 0.03b$	$2890 \pm 4.00a$	$68.30 \pm 0.03b$	$3770 \pm 4.00a$	$76.50 \pm 0.01a$
MTGase	0.10	$2430 \pm 6.00b$	$42.00 \pm 0.08d$	$3783 \pm 5.00b$	$68.90 \pm 0.05e$	$4672 \pm 5.00b$	$77.20 \pm 0.05b$
	0.20	$2493 \pm 8.00c$	$42.30 \pm 0.05e$	$4090 \pm 8.00c$	$68.60 \pm 0.03c$	$4968 \pm 3.50c$	$77.50 \pm 0.03f$
	0.30	$2688 \pm 11.00f$	$42.10 \pm 0.02d$	$4792 \pm 6.00f$	$68.80 \pm 0.02d$	$5878 \pm 9.00f$	$77.50 \pm 0.02e$
	0.40	$2520 \pm 10.00d$	$41.50 \pm 0.06c$	$4470 \pm 10.00e$	$68.80 \pm 0.05d$	$5342 \pm 8.00e$	$77.40 \pm 0.03d$
	0.50	$2552 \pm 11.00e$	$40.20 \pm 0.03a$	$4370 \pm 4.00d$	$68.00 \pm 0.04a$	$5252 \pm 4.00d$	$77.30 \pm 0.01c$

**Table 4.** Maximum temperature ( $T_m$  °C) and enthalpy ( $\Delta H$  J/g) of red tilapia surimi gels prepared using different concentrations of microbial transglutaminase (MTGase)

The numbers represent mean  $\pm$  s.d. of three replications. Different letters in the same column indicate the significant differences ( $P < 0.05$ )

Additive	Levels (%)	Peak 1 (Myosin)		Peak 2 (Actin)	
		$\Delta H$	$T_m$	$\Delta H$	$T_m$
Control	0.00	0.80 $\pm$ 0.03a	41.00 $\pm$ 0.20a	0.72 $\pm$ 0.50a	74.00 $\pm$ 0.10a
MTGase	0.10	2.32 $\pm$ 0.04c	43.30 $\pm$ 0.20b	1.54 $\pm$ 0.06c	74.30 $\pm$ 0.10a
	0.20	2.50 $\pm$ 0.08d	44.50 $\pm$ 0.10d	1.60 $\pm$ 0.03c	74.50 $\pm$ 0.20a
	0.30	2.81 $\pm$ 0.07e	45.00 $\pm$ 0.10e	1.92 $\pm$ 0.08d	75.00 $\pm$ 0.10b
	0.40	1.52 $\pm$ 0.05b	43.70 $\pm$ 0.30c	1.50 $\pm$ 0.02c	74.50 $\pm$ 0.30a
	0.50	1.56 $\pm$ 0.04b	43.50 $\pm$ 0.20c	1.22 $\pm$ 0.05b	74.20 $\pm$ 0.10a

**Fig. 2.** Differential scanning calorimetry thermograms of red tilapia surimi gels affected by different concentrations of microbial transglutaminase; P<sub>1</sub> (myosin peak), P<sub>2</sub> (actin peak).

shown in Table 4. Thermograms of all samples showed two endothermic peaks (Fig. 2), which are related to the thermal denaturation of myosin (P<sub>1</sub>) and actin (P<sub>2</sub>).

Addition of MTGase had significant effect ( $P < 0.05$ ) on  $\Delta H$  and  $T_m$  of the myosin and  $\Delta H$  of actin. Maximum temperature of denaturation for myosin significantly ( $P < 0.05$ ) increased from 43.30  $\pm$  0.20°C to 45.00  $\pm$  0.10°C when the MTGase concentration was increased from 0.10 to 0.30 (units MTGase/g). The  $T_m$  of the actin for surimi gel treated with 0.30 MTGase (units/g surimi) was 75.00  $\pm$  0.10°C, which was significantly higher than that of control surimi gel (74.00  $\pm$  0.10°C), although there were no significant differences ( $P > 0.05$ ) in  $T_m$  of other formulated surimi gels.

There is no significant difference between the enthalpy of myosin in gels with 0.40 and 0.50 MTGase ( $P > 0.05$ ). A significant increase (from 1.54  $\pm$  0.60 to 1.92  $\pm$  0.08) in  $\Delta H$  of actin was observed when the MTGase concentration increased from 0.10 to 0.30. This indicated that the higher MTGase concentration produced more protein denaturation and thus better texture characteristics (Park 2005). Obtained results are in agreement with the findings obtained from breaking force and deformation measurements.

## Conclusion

This study indicated that MTGase addition up to 0.3 units/g surimi can be used to improve red tilapia surimi gel quality. Deformation, breaking force and whiteness increased in surimi gels incorporated with MTGase at 0.1 and 0.2 and 0.3 (units/g surimi), whereas EW and intensity of MHC decreased compared with the control. Storage modulus correlated with the texture and showed an increase in gel elasticity, indicating enhanced thermal gelation of red tilapia surimi. Result of DSC confirmed higher stability of the protein after MTGase treatment. However, protein stability slightly decreased in gels with more than 0.30 MTGase (units/g surimi). The present study contributes additional evidence that suggests the addition of 0.30 MTGase (units/g surimi) to red tilapia surimi gels improved surimi gel quality, whereas exceeding this optimal level did not provide further improvements.

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## References

- Baker KH, Lanier TC, Green DP (2000) Cold restructuring of seafood using transglutaminase mediated binding. In '2000 IFT annual meeting book of abstracts'. pp. 75–76. (IFT Press: Chicago, IL)
- Benjakul S, Visessanguan W (2003) Transglutaminase-mediated setting in bigeye snapper surimi. *Food Research International* **36**, 253–266. doi:10.1016/S0963-9969(02)00167-9
- Benjakul S, Visessanguan W, Riebroy S, Ishizaki S, Tanaka M (2002) Gel-forming properties of bigeye snapper, *Priacanthus tayenus* and *P. macracanthus*, stored in ice. *Journal of the Science of Food and Agriculture* **82**, 1442–1451. doi:10.1002/jsfa.1207
- Benjakul S, Visessanguan W, Thongkaew C, Tanaka M (2005) Effect of frozen storage on chemical and gel forming properties of fish commonly used for surimi production in Thailand. *Food Hydrocolloids* **19**, 197–207. doi:10.1016/j.foodhyd.2004.05.004
- Benjakul S, Julavittayanukul O, Visessanguan W (2006) Effect of phosphate compounds on gel-forming ability of surimi from bigeye snapper (*Priacanthus tayenus*). *Journal of Food Hydrology* **20**, 1153–1163.
- Cardoso C, Mendes R, Vaz-Pires P, Nunes ML (2010) Effect of salt and MTGase on the production of high quality gels from farmed sea bass. *Journal of Food Engineering* **101**, 98–105. doi:10.1016/j.foodeng.2010.06.017
- Chaijan M, Panpipat W (2010) Gel-Forming Ability of Mackerel (*Rastrelliger Branchysoma*) Protein Isolate as Affected by Microbial Transglutaminase. *Walailak Journal of Science and Technology* **7**, 41–49.
- Chanarat S, Benjakul S, H-Kittikun A (2012) Comparative study on protein cross-linking and gel enhancing effect of microbial transglutaminase on surimi from different fish. *Journal of the Science of Food and Agriculture* **92**, 844–852. doi:10.1002/jsfa.4656
- Chen NJ, Morikawa J, Hashimoto T (2005) Effect of amino acids on the eutectic behavior of NaCl solutions studied by DSC. *Cryobiology* **50**, 264–272. doi:10.1016/j.cryobiol.2005.02.004
- Dondero M, Figueroa V, Morales X, Curotto E (2006) Transglutaminase effects on gelation capacity of thermally induced beef protein gels. *Food Chemistry* **99**, 546–554. doi:10.1016/j.foodchem.2005.08.022
- Duangmal K, Taluengphol A (2010) Effect of protein additives, sodium ascorbate, and microbial transglutaminase on the texture and colour of red tilapia surimi gel. *International Journal of Food Science & Technology* **45**, 48–55. doi:10.1111/j.1365-2621.2009.02102.x
- Hemung BO, Chin KB (2013) Effects of fish sarcoplasmic proteins on the properties of myofibrillar protein gels mediated by microbial transglutaminase. *Food Science and Technology (Campinas.)* **53**, 184–190.
- Jiang S-T, Hsieh J-F, Ho M-L, Chung Y-C (2000) Microbial Transglutaminase Affects Gel Properties of Golden Threadfin-bream and Pollack Surimi. *Journal of Food Science* **65**, 694–699.
- Jongjareonrak A, Benjakul S, Visessanguan W, Prodpran T, Tanaka M (2006) Characterization of edible films from skin gelatin of brown stripe red snapper and bigeye snapper. *Food Hydrocolloids* **20**, 492–501. doi:10.1016/j.foodhyd.2005.04.007
- Kaewudom P, Benjakul S, Kijroongrojana K (2012) Effect of bovine and fish gelatin in combination with microbial transglutaminase on gel properties of threadfin bream surimi. *International Aquatic Research* **4**, 12. doi:10.1186/2008-6970-4-12
- Kaewudom P, Benjakul S, Kijroongrojana K (2013) Properties of surimi gel as influenced by fish gelatin and microbial transglutaminase. *Food Bioscience* **1**, 39–47. doi:10.1016/j.fbio.2013.03.001
- Karayannakidis PD, Zotos A, Petridis D, Taylor KDA (2008) The Effect of Washing, Microbial Transglutaminase, Salts and Starch Addition on the Functional Properties of Sardine (*Sardina Pilchardus*) Kamaboko Gels. *Food Science and Technology International* **14**, 167–177.
- Ko WC, Yu CC, Hsu KC (2007) Changes in conformation and sulfhydryl groups of tilapia actomyosin by thermal treatment. *LWT - Food Science and Technology (Campinas.)* **40**, 1316–1320.
- Kuraishi C, Sakamoto J, Yamazaki K, Susa Y, Kuhara C, Soeda T (1997) Production of restructured meat using microbial transglutaminase without salt or cooking. *Journal of Food Science* **62**, 488–490. doi:10.1111/j.1365-2621.1997.tb04412.x
- Kyaw ZY, Cheow CS, Yu SY, Dzulkifly MH (2001) The effect of pressure cooking on the microstructure and expansion of fish cracker (keropok). *Journal of Food Quality* **24**, 181–194. doi:10.1111/j.1745-4557.2001.tb00601.x
- Laemmli UK (1970) Cleavage of structural protein during the assembly of head bacteriophage T4. *Nature* **227**, 680–685. doi:10.1038/227680a0
- Lee CM (1984) Surimi process technology. *Journal of Food Technology* **38**, 69–80.
- Lee HG, Lanier TC, Hamann DD, Knopp JA (1997) Transglutaminase effects on low temperature gelation of fish protein sols. *Journal of Food Science* **62**, 20–24. doi:10.1111/j.1365-2621.1997.tb04359.x
- Lowry QH, Rosebrough NJ, Farr LA, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry* **193**, 256–275.
- Mao L, Wu T (2007) Gelling properties and lipid oxidation of kamaboko gels from grass carp (*Ctenopharynx godonidellus*) influenced by chitosan. *Journal of Food Engineering* **82**, 128–134. doi:10.1016/j.foodeng.2007.01.015
- Norziah MH, Al-Hassan A, Khairulnizam Mordi MN, Norita M (2009) Characterization of fish gelatin from surimi processing wastes: thermal analysis and effect of transglutaminase on gel properties. *Food Hydrocolloids* **23**, 1610–1616. doi:10.1016/j.foodhyd.2008.12.004
- Park JW (2005) Ingredient technology for surimi and surimi seafood. In *Surimi and surimi seafood*. 2nd edn. (Ed. JW Park) pp. 107–140. (CRC Press: Boca Raton, FL, USA).
- Park JW, Lin TM (2005) Surimi: manufacturing and evaluation. In *Surimi and Surimi Seafood*. (Ed. JW Park) pp. 33–105. (Taylor and Francis: Boca Raton, FL, USA).
- Ramírez JA, Rodríguez-Sosa R, Morales OJ, Vázquez M (2000) Surimi gels from striped mullet (*Mugil cephalus*) employing microbial transglutaminase. *Food Chemistry* **70**, 443–449. doi:10.1016/S0308-8146(00)00109-6
- Ramírez JA, Velázquez G, Echevarría GL, Torres JA (2007) Effect of adding insoluble solids from surimi wash water on the functional and mechanical properties of Pacific Whiting grade A surimi. *Bioresource Technology* **98**, 2148–2153. doi:10.1016/j.biortech.2006.08.024
- Rawdkuen S, Benjakul S, Visessanguan W, Lanier TC (2008) Rheological and textural properties of pacific whiting surimi gels as influenced by chicken plasma. *International Journal of Food Properties* **11**, 820–832. doi:10.1080/10942910701624744
- Rawdkuen S, Sai-Ut S, Khamorn S, Chaijan M, Benjakul S (2009) Biochemical and gelling properties of tilapia surimi and protein recovered using an acid alkaline process. *Food Chemistry* **112**, 112–119. doi:10.1016/j.foodchem.2008.05.047
- Shie JS, Park JW (1999) Physical characteristics of surimi seafood as affected by thermal processing conditions. *Journal of Food Science* **64**, 287–290. doi:10.1111/j.1365-2621.1999.tb15884.x
- Spink CH (2008) Differential scanning calorimetry. *Methods in Cell Biology* **84**, 115–141. doi:10.1016/S0091-679X(07)84005-2
- Tammattinna A, Benjakul S, Visessanguan W, Tanaka M (2007) Gelling properties of white shrimp (*Penaeus vannamei*) meat as influenced by setting condition and microbial transglutaminase. *LWT- Food Science and Technology (Campinas.)* **40**, 1489–1497.
- Thorarindottir KA, Arason S, Geirsdottir M, Bogason SG, Kristbergsson K (2002) Changes in myofibrillar proteins during processing of salted cod (*Gadus morhua*) as determined by electrophoresis and differential

- scanning calorimetry. *Food Chemistry* **77**, 377–385. doi:10.1016/S0308-8146(01)00349-1
- Xiong YL (1997) Structure-functionality relationships of muscle proteins. In 'Food proteins and their applications'. (Eds S Damoradan, A Paraf) pp. 341–392. (Marcel Dekker, Inc.: New York, NY)
- Yetim H, Ockerman HW (1995) The effects of egg white, tumbling and storage time on proximate composition and protein fractions of restructured fish products. *Journal of Aquatic Food Product Technology* **4**, 65–78. doi:10.1300/J030v04n01\_05
- Yin T, Park JW (2014) Effects of nano-scaled fish bone on the gelation properties of Alaska pollock surimi. *Food Chemistry* **150**, 463–468.
- Yokoyama K, Nio N, Kikuchi Y (2004) Properties and applications of microbial transglutaminase. *Applied Microbiology and Biotechnology* **64**, 447–454. doi:10.1007/s00253-003-1539-5
- Yongsawatdigul J, Piyadhamviboon P (2005) Effect of microbial transglutaminase on autolysis and gelation of lizardfish surimi. *Journal of the Science of Food and Agriculture* **85**, 1453–1460. doi:10.1002/jsfa.2149
- Yongsawatdigul J, Worratao A, Park JW (2002) Effect of endogenous transglutaminase on threadfin bream surimi gelation. *Journal of Food Science* **67**, 3258–3263. doi:10.1111/j.1365-2621.2002.tb09575.x
- Zhou A, Benjakul S, Pan K, Gong J, Liu X (2006) Cryoprotective effects of trehalose and sodium lactate on tilapia (*Sarotherodon nilotica*) surimi during frozen storage. *Food Chemistry* **96**, 96–103. doi:10.1016/j.foodchem.2005.02.013