

Effects of pH-Shift Processing and Microbial Transglutaminase on the Gel and Emulsion Characteristics of Porcine Myofibrillar System

Geun-Pyo Hong¹, Ji-Yeon Chun², Yeon-Ji Jo², and Mi-Jung Choi*

Department of Bioresources and Food Science, Konkuk University, Seoul 143-701, Korea

¹Department of Food Bioengineering, Jeju National University, Jeju 690-756, Korea

²Department of Bioindustrial Technologies, Konkuk University, Seoul 143-701, Korea

Abstract

This study investigated the effects of microbial transglutaminase (MTGase) and pH-shift processing on the functional properties of porcine myofibrillar proteins (MP). The pH-shift processing was carried out by decreasing the pH of MP suspension to 3.0, followed by re-adjustment to pH 6.2. The native (CM) and pH-shifted MP (PM) was reacted with and without MTGase, and the gelling and emulsion characteristics were compared. To compare the pH-shifted MTGase-treated MP (PT), deamidation (DM) was conducted by reacting MTGase with MP at pH 3.0. Rigid thermal gel was produced by MTGase-treated native MP (CT) and PT. PM and DM showed the lowest storage modulus (G') at the end of thermal scanning. Both MTGase and pH-shifting produced harder MP gel, and the highest gel strength was obtained in PT. All treatments yielded lower than CM, and CT showed significantly higher yield than PM and DM treatments. For emulsion characteristics, pH-shifting improved the emulsifying ability of MP-stabilized emulsion, while the treatments had lower emulsion stability. PM-stabilized emulsion exhibited the lowest creaming stability among all treatments. The emulsion stability could be improved by the usage of MTGase. The results indicated that pH-shifting combined with MTGase had a potential application to modify or improve functional properties of MP in manufacturing of meat products.

Key words: transglutaminase, myofibrillar, pH-shifting, deamidation, functional properties

Introduction

Myofibrillar protein (MP) plays key role in thermal MP gel formation as well as texturization of meat products. Because of salt-soluble nature of MP, reducing salt concentration in meat products negatively affect the textural properties of final products (Hamm, 1986). To improve rheological and textural properties of meat products, various techniques have been introduced including the addition of protein or carbohydrate-based binding agents, the usage of protein cross-linking enzymes as well as high pressure processing (Chin *et al.*, 2009; Hong *et al.*, 2012).

The pH-shift process is an alternative technique to improve functional properties of proteins. The concept of this processing is based on the protein recovery at either acidic or alkaline conditions (Tadpichayangkoon *et al.*, 2010). At extreme pH conditions, net surface charges of proteins

are enhanced and the proteins undergo unfolding. By re-adjusting pH at near neutral region, proteins are refolded, while conformational changes of the proteins occur including exposures of hydrophobic sites and sulfhydryl groups (Kristinsson and Hultin, 2003). In the case of MP, dissociation or partial hydrolysis of myosin heavy chain is reported and these changes in MP conformation lead to better functional properties in forming a gel or stabilizing emulsion (Jiang *et al.*, 2009; Tadpichayangkoon *et al.*, 2010).

Microbial transglutaminase (MTGase) is an enzyme which polymerizes the proteins by promoting acyl transfer reaction between γ -carboxamide group of glutamine residue and ϵ -amino group of lysine (Kuraish *et al.*, 1997). Owing to its potential advantages in cold-set protein gelation, MTGase has been extremely investigated in the production of semi-solid protein-based foods such as processed meat, surimi, cheese and tofu (Jiang *et al.*, 2009; Kuraish *et al.*, 1997; Pérez-Mateos and Lanier, 2006). As a consequence of MTGase action, inter- and intra-protein interactions are dominated, which results in loss of protein solubility (Chin *et al.*, 2009). Meanwhile, some liter-

*Corresponding author: Mi-Jung Choi, Department of Bioresources and Food Science, Konkuk University, Seoul 143-701, Korea. Tel: 82-2-450-3048, Fax: 82-2-455-1044, E-mail: choimj@konkuk.ac.kr

ature reported different pattern of MTGase reactions where the MTGase treated substrate proteins had better hydration ability and functional properties (Hong and Xiong, 2012). It was known that acyl transfer reaction involved in transamidation (cross-linking) and deamidation, and the deamidation was occurred under the absence of free amines (Orrù *et al.*, 2003). The deamidation indicates that γ -carboxamide group (Gln-CONH₂) of glutamine residue is converted to carboxyl group (Glu-COOH) and the new formation of carboxyl groups maybe attributed better functional properties (Orrù *et al.*, 2003; Trevino *et al.*, 2007).

To manifest deamidation, pH of proteins has to be lowered to below isoelectric point which is a part of pH-shift process. The pH-shifting and deamidating processes are depending on the point of time when MTGase is reacted. Although, a few researches have been conducted to estimate the functional properties of pH-shifted proteins, there is no information about the functional properties of pH-shift combined with MTGase-treated MP. Therefore, this study investigated the effects of pH-shifting and microbial transglutaminase on the gelation and emulsion properties of porcine MP and compared with deamidated MP.

Materials and Methods

Materials

Pork loins (*m. longissimus dorsi*) with pH 5.5-5.7 were obtained from 24 h post-mortem carcasses. The meat was trimmed of visible fat and connective tissue, and cut into 1 cm cubes. Approximately 200 g portions of the cubes were vacuum packaged using a poly nylon pouch and frozen at -50°C prior to use (within 3 wk). MTGase (Activati, 1% enzyme and 99% maltodextrin) was donated by Ajinomoto Food Ingredient (USA). All chemicals used in the present study were reagent grade.

MP extraction

The MP was extracted by removing sarcoplasmic proteins (Xiong, 1992) with minor modification (Chin *et al.*, 2009). Frozen meat cubes were thawed at 4°C overnight. Minced meat was washed three times with 4 vol. (v/w) of 0.1 M NaCl in 50 mM sodium phosphate buffer (pH 6.2) followed by 8 vol. (v/w) of 0.1 M NaCl (pH 6.2). The final MP suspension was filtered using two layers of gauze and adjusted pH to 6.2 by adding 1 M HCl. Protein concentration of extracted MP was determined by Biuret method (Gornall *et al.*, 1949) using a Kjeldahl protein concentration calibrated bovine serum albumin (BSA) as

a standard. The MP was stored on ice prior to use (within 24 h).

Sample preparation

MP (50 mg/mL protein) and enzyme (3%, w/w) stocks were prepared by respectively suspending MP and enzyme with 0.3 M NaCl (final concentration basis) in 25 mM cold sodium citrate buffer (pH 6.2). The control MP suspension was prepared by mixing MP stock with 25 mM sodium citrate buffer (0.3 M NaCl, pH 6.2) to provide final 40 mg/mL protein concentration (CM), while the citrate buffer was replaced with the enzyme stock for the preparation of MTGase treatment (CT, 40 mg/mL protein with 0.3% enzyme). The pH-shifting was applied by decreasing the pH of MP stock down to 3.0 using 1 M HCl, holding at 4°C for 2 h, thereafter increasing pH up to 6.2 using 1 M NaOH (Jiang *et al.*, 2009). After that, half of the pH-shifted MP was adjusted to 40 mg/mL protein (PM) while the remaining was reacted with MTGase (PT, 40 mg/mL protein with 0.3% enzyme) as the same procedure described above. To prepare deamidated MP (DM), pH of the MP stock was lowered to pH 3.0 and mixed with MTGase (final 0.3%, w/w) in 25 mM sodium citrate buffer containing 0.3 M NaCl (pH 3.0). The mixture was further hold at 4°C for 2 h, thereafter sample pH was adjusted to 6.2 with protein concentration of 40 mg/mL.

Gelation characteristics

All samples were centrifuged at 700 g for 30 s to remove air cells. The viscoelastic moduli of sample were determined within the linear viscoelastic region using an oscillatory rheometer (Bohlin Instruments, Inc., USA) equipped with a 40 mm parallel plate in diameter. The sample suspension was loaded onto the sample plate and covered with paraffin oil to prevent evaporative loss. Sample was equilibrated at 20°C for 3 min and heated from 20 to 80°C at 1°C/min. The measurement was made at a fixed frequency of 0.1 Hz with strain amplitude of 0.02. The storage modulus (G') was recorded throughout thermal scanning and repeated twice per treatment. For puncture test, 5 g sample aliquots were loaded into six glass tubes (15 mm diameter). The samples were incubated at 4°C for 24 h, and thermal treated from 4 to 80°C at 1°C/min using a programmable water bath. The heated samples were cooled in ice for 10 min and tempered at ambient for 1 h. Before puncturing, exudates were discarded and the weights of the sample were recorded to estimate yield. Yield of MP was calculated by percentage sample weight over the initial weight. The samples were

then compressed with an Instron testing machine (Instron Corporation, USA) with a plunger (9 mm diameter) and 50 mm/min head speed. The force at the failure (the first peak) was expressed as gel strength.

Emulsion characteristics

MP-stabilized corn oil-in-water emulsion was prepared by diluting 6 g sample suspension with 21 g of 0.3 M NaCl in 50 mM sodium phosphate buffer (pH 6.25). The diluted suspension was incubated at 4°C for 24 h, and then 3 g corn oil was added. This formulation allowed 10 mg/mL protein, 0.075% (w/w) enzyme concentration and 10% (w/w) corn oil. The mixture was homogenized for 1 min with an Ultra-Turrax (T25 basic, Ika Works Inc., USA) at 13,000 rpm. For cream stability, 10 g aliquots were transferred to glass tube (1 cm diameter) immediately after homogenization. After standing 30 min at 4°C, the height ratio of serum layer was calculated and creaming index was expressed as a percentage height over total height (Ionescu *et al.*, 2008). Emulsification activity and emulsion stability indices were determined by the method of Pearce and Kinsella (1978). From the emulsion dispersed sample, 30 μ L aliquots were taken at 0 and 10 min, respectively and diluted with 6 mL of 0.1% (w/v) sodium dodecyl sulfate (SDS) solution. The absorbance at 500 nm was measured and the turbidity (T) was calculated by

$$T = \frac{2.303 \times A}{L}$$

where A is the absorbance and L is the path length of the cuvette (1 cm). The emulsification activity index (EAI) was numerically calculated by

$$EAI(m^2/g) = \frac{2 \times T \times D}{C(1-\phi) \times 10^4}$$

where D , C and ϕ indicated dilution factor (201), oil volume fraction (0.093) and protein concentration (0.01 g/mL), respectively. The emulsion stability index (ESI) was determined by

$$ESI(\%) = \frac{T}{T_0} \times 100$$

where T_0 and T indicated the turbidity at time 0 and 10 min, respectively. The microstructure of emulsion was observed after standing the emulsion dispersed sample at 4°C for 0 and 1 h, respectively, using an optical microscope (Eclipse E400, Nikon Corporation, Japan) connected to a CCD camera (CCD-300 T-RC, DAGE-MTI., USA).

Statistical analysis

A completely randomized design was adopted to analyze the effects of various MP treatments. All treatment effects were tested three times on different days using a new batch of MP preparation ($n=3$). One way analysis of variance (ANOVA) was performed using a SAS (ver. 9.1) and the means were separated by Duncan's multiple range test when the treatment effects were significant ($p<0.05$).

Results and Discussion

Gelling characteristics

Thermal gelling behavior of CM exhibited general pattern of MP, where the G' steeply increased at 43°C and showed a peak at 53°C, thereafter decreased drastically (Fig. 1). Xiong and Blanchard (1994) reported that dissociation of myofibrils indicated the decrease in G' at 50–55°C. The G' increased again from 54°C which was resulted from an irreversible network formation (Xiong and Blanchard, 1994). The G' of PM showed similar pattern to CM as showing a peak at around 56°C, thereafter the G' increased slightly until the end of thermal scanning. The extent of G' increase at the temperature above 56°C was not high in PM comparing to CM, reflecting lower gelling ability of PM than that of CM. The result was not in consistency with Kristinsson and Liang (2006) who postulated better gelling ability of pH-shifted Atlantic croaker muscle proteins. Meanwhile, Ingadottir and Kristinsson (2010) reported that pH-shifted tilapia muscle showed lower G' after thermal treatment than control. The different results were possibly due to experimental conditions such as protein sources, pH and NaCl, whereas dissociation of myosin heavy chain during pH-shifting

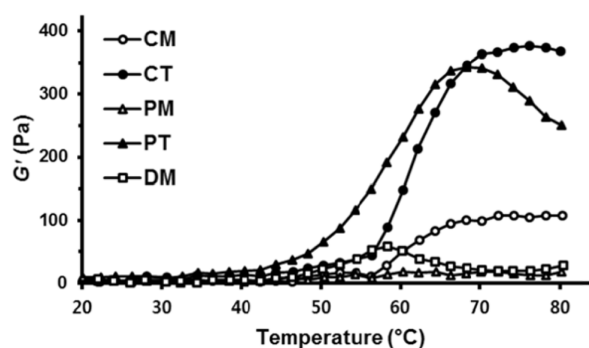


Fig. 1. Effects of pH-shifting and microbial transglutaminase (MTGase) on the storage modulus (G') of porcine myofibrillar proteins (MP). CM, control MP; CT, MTGase-treated CM; PM, pH-shifted MP; PT, MTGase-treated PM; DM, deamidated MP.

would result in less rigidity of thermal induced MP gelation (Kristinsson and Hultin, 2003). In the case of DM, maximum G' was estimated at 58°C thereafter the G' was gradually decreased with increasing temperature. It has been reported that hydrophobic and electrostatic interactions were involved in thermal MP gel formation (Hamm, 1986). During pH-shift processing, MP was partially unfolded and aggregated (Tadpitchayangkoon *et al.*, 2010) which would account for the lower G' of PM and DM treatments at the end of heating. Consequently, the pre-treatments of MP (pH-shifting or deamidation) attributed to less rigid thermal gel formation comparing to native MP.

For MTGase reaction, CT started a steady increase in G' at 39°C and the G' increased drastically at 54°C. The result was also identical in our previous study (Hong and Xiong, 2012). An evidence of peak during thermal scanning was not shown in CT, reflecting that fluidity caused from dissociated myosin light chains might not be occurred in CT due to cross-linking of MP by MTGase. The pattern of G' change in CT was also similar to PT, although the drastic increase in G' of PT was found at relatively lower temperature (50°C). In addition, the G' decreased at heating temperature higher than 68°C, indicating that structural modification of pH-shifted MP might affect different reaction with MTGase.

Gel strength of MP was increased not only by addition of MTGase but also pH-shifting process and the pre-treatments affected yield of MP gel (Fig. 2). The PM treatment had significantly higher gel strength than CM, but lower than CT ($p < 0.05$). The pH-shifting combined with MTGase treatment (PT) had 28.6 g_f of the highest gel strength among all treatments ($p < 0.05$). Meanwhile DM showed the similar gel strength to CT and PM treatments. In the present study, hard gel formation of MTGase-treated MP (CT and PT) was in agreement with the report of Pérez-Mateos and Lanier (2006) and was regarded as being appropriate because of protein cross-linking. On the other hand, harder gel formation of PM than CM was interesting, because PM exhibited less rigid thermal gelation (characterized by low G') comparing to CM. Rawdkuen *et al.* (2009) reported that acid processed tilapia muscle exhibited lower gel strength than that of conventional washed muscle. However, they compared muscle proteins isolated from different methods, hence, acid-aided muscle contained more amount of sarcoplasmic fractions than conventional processed muscle. On the other hand, Ingadottir and Kristinsson (2010) reported that pH-shifting produced harder MP muscle protein gel due to conformational changes

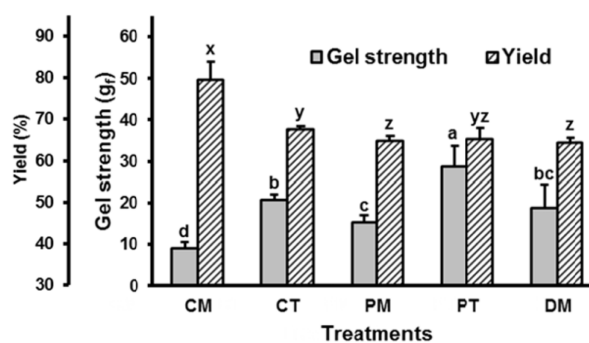


Fig. 2. Effects of pH-shifting and microbial transglutaminase (MTGase) on the gel strength and yield of porcine myofibrillar proteins (MP). Vertical bars indicate standard deviation ($n=3$). Means with different letters (a-d, x-z) are significantly different ($p < 0.05$). CM, control MP; CT, MTGase-treated CM; PM, pH-shifted MP; PT, MTGase-treated PM; DM, deamidated MP.

in MP. Tadpitchayangkoon *et al.* (2010) noted that changes in disulfide formation, hydrogen bonding and hydrophobic interactions by pH-shifting attributed harder protein gel formation. Meanwhile, deamidation indicated the conversion of glutamine to glutamic acid, hence hydrogen bonding was predominant during cooling due to formation of carboxyl group (Hong and Xiong, 2012). This change in side residues of MP would provide harder gel than CM, which warranted further exploration.

For protein-water interactions, yield of MP was about 80% which was highest among all treatments, reflecting the pre-treatments of MP attributed the water-binding properties of MP negatively. Although CT had significantly higher yield than those of PM and DM ($p < 0.05$), all treatments showed 65-68% yield. It was expected that MTGase promoted better protein-protein interactions which made weak protein-water interactions (Chin *et al.*, 2009). However, it was intriguing that pH-shifting and deamidation reduced the yield of MP gel because dissociation of myosin heavy chain in the former and carboxyl group formation in the latter could be related to better water-binding properties of MP (Hong and Xiong, 2012; Kristinsson and Hultin, 2003). Possibly, pH-shifting process including deamidating procedure caused partial denaturation of MP which reduced protein solubility and hydration ability (Tadpitchayangkoon *et al.*, 2010). As a result, all treatments would display lower yield than that of native control (CM). From the result, pH-shifting combined with MTGase treatment was supposed to be an alternative method to improve rheological and textural properties of MP.

Emulsion characteristics

Emulsion properties characterized by indices of MP stabilized emulsions are presented in Fig. 3. The EAI of CM was significantly higher than that of CT, but lower than pH-shifted samples ($p < 0.05$). CT had low solubility due to polymerization of MP which caused bridging flocculation and led to relatively poor emulsifying ability (Dickinson, 2003; Hong *et al.*, 2012). Meanwhile, better emulsifying abilities of pH-shifted proteins have been identical in previous reports (Jiang *et al.*, 2009). MP revealed more hydrophobic sites from the interior of the MP structure at acidic condition. Upon re-adjusting pH to near neutral, the MP is refolded with maintaining aliphatic and aromatic hydrophobic sites (Tadpichayangkoon *et al.*, 2010). Another contribution for better emulsifying capacity of pH-shifted MP is dissociation (or partial hydrolysis) of myosin heavy chain (Kristinsson and Hultin, 2003). By decreasing the molecular weight, MP enabled to be adsorbed on the interface of oil droplets effectively, and as a consequence, it attributed better emulsifying ability. It was thought that the DM also affected emulsification as the same mechanism of pH-shifted MP since deamidated MP showed higher hydrophobicity as well as an evidence of partial hydrolysis of myosin heavy chain (Hong and Xiong, 2012). In contrast, the ESI showed an opposite result with the EAI. Regardless of MTGase treatment, all pH-shifted MP including DM had lower EAI than those of native MP ($p < 0.05$). The poor emulsion stability of pH-shifted MP would be resulted from a partial denaturation during process. In the meantime, no influence of MTGase on the ESI of MP-stabilized emulsion would be due to short

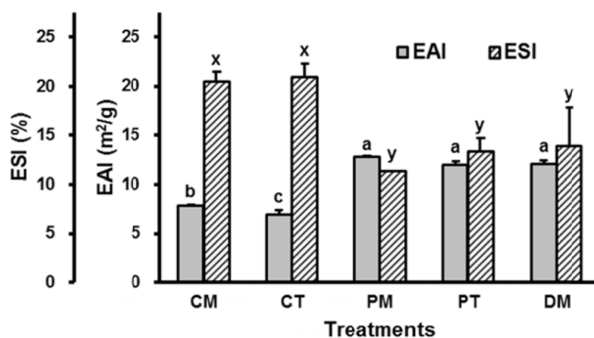


Fig. 3. Effects of pH-shifting and microbial transglutaminase (MTGase) on the emulsification activity index (EAI) and emulsion stability index (ESI) of porcine myofibrillar proteins (MP)-stabilized emulsions. Vertical bars indicate standard deviation ($n=3$). Means with different letters (a-c, x-y) are significantly different ($p < 0.05$). CM, control MP; CT, MTGase-treated CM; PM, pH-shifted MP; PT, MTGase-treated PM; DM, deamidated MP.

incubation time (10 min at 4°C) and low enzyme concentration (0.075%). Although, the MTGase promoted a MP cross-linking as shown in lower EAI of CT-stabilized emulsion, the impact of the enzyme on the rheological properties of continuous phase of the emulsion system was not pronounced, thereby causing no effect on the emulsion stability. To verify the effects of MTGase and pH-shift process on the stability of MP-stabilized emulsion, cream stability was also compared (Fig. 4). The impacts of factors (enzyme and pre-treatments) on the emulsion stability were further separated in creaming index. Still native MP exhibited higher creaming stability than pH-shifted MP, CT displayed better emulsion stability than CM ($p < 0.05$). During incubation, MTGase increased the apparent viscosity of continuous phase of the emulsion which delayed the phase separation (Hong *et al.*, 2012). Ramírez-Suárez and Xiong (2003) noted that the MTGase-mediated cross-linking strengthened the interfacial layer of emulsion droplets and prevented coalescence by reducing the flexibility of the MP. For the same mechanism, PT showed significantly high creaming index than PM ($p < 0.05$). DM showed higher creaming index than PM, but lower than PT ($p < 0.05$). There was no evidence of MTGase action (cross-linking) after re-adjusting pH in DM because of no G' increment during thermal scanning (Fig. 1). The actual mechanisms involved in MTGase-mediated deamidation on the emulsion characteristics remained still obscure which warranted further exploration.

The microstructure of MP-stabilized emulsion supported the findings in MP-stabilized emulsion characteristics (Fig. 5). Initially (at 0 h), native MP showed bigger droplets than those prepared by pH-shifted MP. After incubation for

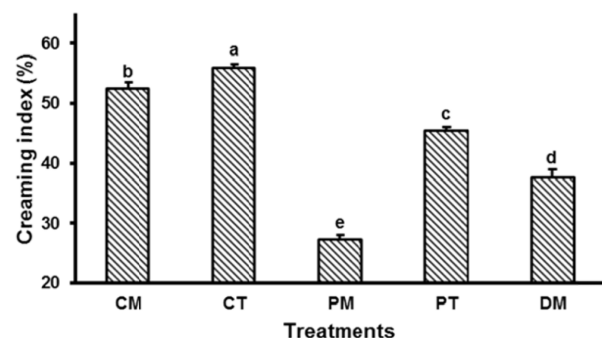


Fig. 4. Effects of pH-shifting and microbial transglutaminase (MTGase) on the creaming index of porcine myofibrillar proteins (MP)-stabilized emulsions. Vertical bars indicate standard deviation ($n=3$). Means with different letters (a-e) are significantly different ($p < 0.05$). CM, control MP; CT, MTGase-treated CM; PM, pH-shifted MP; PT, MTGase-treated PM; DM, deamidated MP.

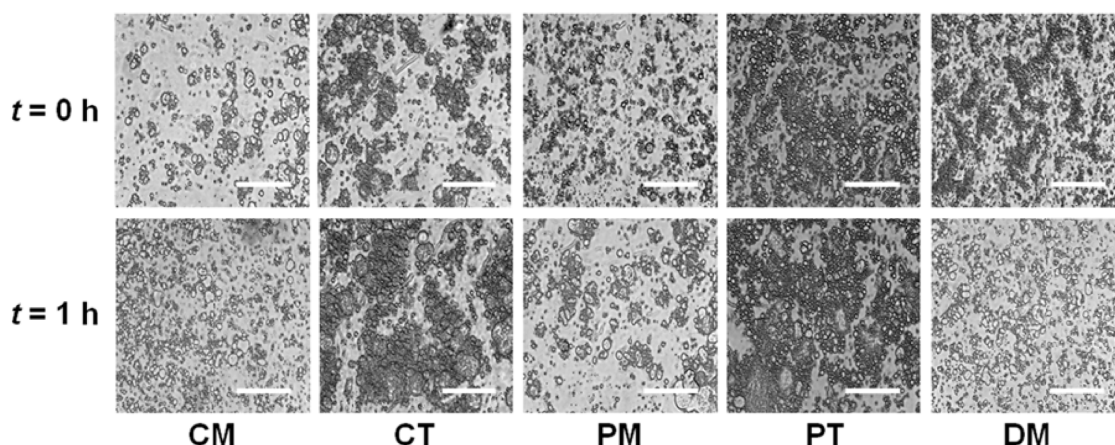


Fig. 5. Microstructure of 10% corn oil-in-water emulsion stabilized by porcine myofibrillar proteins (MP) after emulsification of 0 and 1 h, respectively. Scale bars indicate 20 μm . CM, control MP; CT, MTGase-treated CM; PM, pH-shifted MP; PT, MTGase-treated PM; DM, deamidated MP.

1 h, increases in droplet sizes were observed in all treatments, particularly the increase in droplet size of PM-stabilized emulsion was considerable, reflecting intensive coalescence was occurred in PM. On the other hand, MTGase treatments showed an aggregation among emulsion droplets and formed a continuous network structure. This would account for better stability of MTGase-treated emulsion as described above. Consequently, the results indicated that pH-shifting of MP did not improve emulsion properties due to its poor emulsion stabilizing nature, nonetheless the usage of MTGase could minimize the drawback of destabilization of MP-stabilized emulsion.

References

- Chin, K. B., Go, M. Y., and Xiong, Y. L. (2009) Konjac flour improved textural and water retention properties of transglutaminase-mediated, heat-induced porcine myofibrillar protein gel: Effect of salt level and transglutaminase incubation. *Meat Sci.* **81**, 565-572.
- Dickinson, E. (2003) Hydrocolloids at interface and the influence on the properties of dispersed systems. *Food Hydrocolloid.* **17**, 25-39.
- Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**, 751-766.
- Hamm. (1986) Functional properties of the myofibrillar system and their measurements. In: Muscle as food. P. J. Bechtel (ed.), pp. 135-199. New York: Academic Press, Inc.
- Hong, G. P., Min, S. G., and Chin, K. B. (2012) Emulsion properties of pork myofibrillar protein in combination with microbial transglutaminase and calcium alginate under various pH conditions. *Meat Sci.* **90**, 185-193.
- Hong, G. P., and Xiong, Y. L. (2012) Microbial transglutaminase-induced structural and rheological changes of cationic and anionic myofibrillar proteins. *Meat Sci.* **91**, 36-42.
- Ingadottir, B. and Kristinsson, H. G. (2010) Gelation of protein isolates extracted from tilapia light muscle by pH shift processing. *Food Chem.* **118**, 789-798.
- Ionescu, A., Aprodu, I., Darabă, A., and Porneală, L. (2008) The effects of transglutaminase on the functional properties of the myofibrillar protein concentrate obtained from beef heart. *Meat Sci.* **79**, 278-284.
- Jiang, J., Chen, J., and Xiong, Y. L. (2009) Structural and emulsifying properties of soy protein isolates subjected to acid and alkaline pH-shifting processes. *J. Agric. Food Chem.* **57**, 7576-7583.
- Kristinsson, H. G. and Hultin, H. O. (2003) Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. *J. Agric. Food Chem.* **51**, 7187-7196.
- Kristinsson, H. G. and Liang, Y. (2006) Effect of pH-shift processing and surimi processing on Atlantic croaker (*Micropogonias undulates*) muscle proteins. *J. Food Sci.* **71**, C304-C312.
- Kuraish, C., Sakamoto, K., Yamazaki, K., Susa, Y., Kuhara, C., and Soeda, T. (1997) Production of restructured meat using microbial transglutaminase without salt or cooking. *J. Food Sci.* **62**, 488-490, 515.
- Orrù, S., Caputo, I., D'Amato, A., Ruoppolo, M., and Esposito, C. (2003) Proteomics identification of acyl-acceptor and acyl-donor substrates for transglutaminase in a human intestinal epithelial cell line. *J. Biol. Chem.* **278**, 31766-31773.
- Pearce, K. N., and Kinsella, J. E. (1978) Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **26**, 716-723.
- Pérez-Mateos, M. and Lanier, T. C. (2006) Comparison of Atlantic menhaden gels from surimi processed by acid or alkaline solubilisation. *Food Chem.* **101**, 1223-1229.
- Ramírez-Suárez, J. C. and Xiong, Y. L. (2003) Rheological

- properties of mixed muscle/nonmuscle protein emulsions treated with transglutaminase at two ionic strengths. *Int. J. Food Sci. Technol.* **38**, 777-785.
17. Rawdkuen, S., Sai-Ut, S., Khamorn, S., Chaijan, M., and Benjakul, S. (2009) Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process. *Food Chem.* **112**, 112-119.
 18. Tadpitchayangkoon, P., Park, J. W., Mayer, S. G., and Yongsawatdigul, J. (2010) Structural changes and dynamic rheological properties of sarcoplasmic proteins subjected to pH-shift method. *J. Agric. Food Chem.* **58**, 4241-4249.
 19. Trevino, S. R., Scholtz, J. M., and Pace, C. N. (2007) Amino acid contribution to protein solubility: Asp, Glu, and Ser contribute more favorable than the other hydrophilic amino acids in RNase Sa. *J. Mol. Biol.* **366**, 449-460.
 20. Xiong, Y. L. (1992) A comparison of the rheological characteristics of different fractions of chicken myofibrillar proteins. *J. Food Biochem.* **16**, 217-227.
 21. Xiong, Y. L. and Blanchard, S. P. (1994) Myofibrillar protein gelation: viscoelastic changes related to heating procedures. *J. Food Sci.* **59**, 734-738.

(Received 2013.12.31/Revised 2014.3.10/Accepted 2014.3.14)