

## Evaluation of Porcine Myofibrillar Protein Gel Functionality as Affected by Microbial Transglutaminase and Red Bean [*Vigna angularis*] Protein Isolate at Various pH Values

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### Abstract

This study was investigated to determine the effect of microbial transglutaminase (MTG) with or without red bean protein isolate (RBPI) on the porcine myofibrillar protein (MP) gel functionality at different pH values (pH 5.75-6.5). Cooking yield (CY, %), gel strength (GS, gf), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM) were determined to measure gel characteristics. Since no differences were observed the interaction between 1% RBPI and pH, data were pooled. CY increased with the addition of 1% RBPI, while it was not affected by pH values. GS increased with increased pH and increased when 1% RBPI was added, regardless of pH. There were distinctive endothermic protein peaks, at 56.55 and 75.02°C at pH 5.75, and 56.47 and 72.43 °C at pH 6.5 in DSC results, which revealed decreased temperature of the first peak with the addition of 1% RBPI and increased pH. In SEM, a more compact structure with fewer voids was shown with the addition of 1% RBPI and increased pH from 5.75 to 6.5. In addition, the three-dimensional structure was highly dense and hard at pH 6.5 when RBPI was added. These results indicated that the addition of 1% RBPI at pH 6.5 in MTG-mediated MP represent the optimum condition to attain maximum gel-formation and protein gel functionality.

**Keywords:** red bean protein isolate, myofibrillar protein gel functionality, microbial transglutaminase, pH.

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### Introduction

Myofibrillar protein is crucial for improved textural properties of processed meats (Sun and Holley, 2011). The myosin and actin components of myofibrillar protein promote and contribute to the formation of the desired gel during heating. This processing increases water retention to form a tertiary structure of a protein (Yasui *et al.*, 1979) in which combined emulsifying fat and moisture affect the flavor, texture, cohesion, and yield of the final product after heating (Doerscher *et al.*, 2003; Lee and Chin, 2010; Muguruma *et al.*, 2003).

Interaction of muscle proteins involves disulfide bonds generated from myosin tail section and helix-coil transition as a result of protein cross-linking, in which formation of partially denatured proteins occurs due to the aggregation of the myosin head (Samejima *et al.*, 1981;

Sharp and Offer, 1992). Physicochemical properties of myosin and salt-soluble proteins can be affected by various temperature and pH conditions during heating (Egeland *et al.*, 1986). Therefore, understanding of physicochemical and functional properties of muscle protein, as affected by pH, salt concentration, protein content, and heating, is necessary to modify the interactions between myosin and actin, and other non-meat proteins during the heat-induced gelation process. The combination of myofibrillar proteins and fats, and transglutaminase with or without non-meat proteins significantly affect the gel texture (Sun and Holley, 2011).

In particular, properties of protein gels depend on pH, which promotes the water holding capacity of muscle protein. The isoelectric point of myofibrillar protein is approximately pH 5.4-5.5 and the optimum pH of heat-treated protein is approximately 6.0 (Sun and Holley, 2011). In the absence of heat, protein could be coagulated at acidic pH (Sun and Holley, 2011).

The interaction between pH and the muscle protein condition is important with respect to the physicochemical and functional properties of meat during processing.

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The pH level determines the number of negatively or positively charged groups as pH diverges from the isoelectric point (pI) of the muscle proteins, and directly affects the electrostatic force that acts to pull the water molecules in the reaction between protein structures. In general, as pH increases, the number of negatively charged carboxyl groups increases, whereas the positive charges of the amino groups are increased as pH decreases. The pH of the post-mortem raw meat used for meat processing is around 5.5 to 6.0. Processing at lower pH may decrease the water holding capacity of the final product (Park *et al.*, 2003).

A study investigated the role of pH on gel formation of low sodium chicken muscle proteins reported that myofibrillar protein gel properties were dependent on pH, with the best gel-forming ability at neutral pH (Chang *et al.*, 2001). An evaluation of the gel properties of chicken breast meat proteins at acidic pH and low sodium content reported that pH 3.16 was better for gel formation than pH 3.70 or 4.06, with protein solubility being greater at pH 3.78 (86.3%) than at pH 4.45 (5.2%) (Ke and Hultin, 2005). pH contributes to the formation of secondary structure in porcine myofibrillar protein, with protein interaction being is dependent on pH and temperature, since increased pH reduces gel strength and increases water holding capacity (Liu *et al.*, 2008). Liu *et al.* (2008) also reported that increased  $\beta$ -sheet content in protein weaken the water holding capacity and a pH of 6.5 was needed to form a solid gel.

Protein characteristics and gel functionality as affected by various pH level, and/or non-meat proteins with or without microbial transglutaminase (MTG) have been studied with the goal of optimizing meat processing conditions (Agyare *et al.*, 2008, 2009; Arogundade *et al.*, 2006; Westphalen *et al.*, 2005, 2006). A previous study described the high protein solubility and emulsion stability of red bean protein isolate (RBPI) at various pHs (Meng and Ma, 2002a). In our previous study, RBPI improve the gelling properties and cooking yield of meat protein gels mediated by MTG, which could improve the textural and functional properties of meat proteins as a potential water and meat binder (Jang and Chin, 2011; Jang *et al.*, 2015), which could improve the textural properties of meat products (Hong and Chin, 2010). In our previous study, Jang *et al.* (2015) reported that the optimum condition was 1% RBPI induced with 1% MTG. However, the interaction of RBPI addition in the protein mixture as affected by pH condition was not fully understood. Thus, the objective of this study was to evaluate the effect of MTG combined with or without RBPI on the functionality of porcine MP

gels at different pH values (pH 5.75-6.5).

## Materials and Methods

### Heat-induced gelation

Pork loins (crossbred pigs, (Landrace  $\times$  Large Yorkshire)  $\times$  Duroc; grade A, 110 kg live weight) purchased from a local meat market in Gwangju, Korea, were prepared by cutting the trimming of excess fat and connective tissues, and vacuum packaging, 200 g meat cubes (1 cm<sup>3</sup>). The meat cubes were homogenized for extraction of the myofibrillar protein isolates (MP) by washing with a buffer solution comprised of 0.1 M NaCl and 50 mM NaH<sub>2</sub>PO<sub>4</sub> (pH 6.25) and centrifugation at 1,000 g for 15 min, as slightly modified by a previous description (Xiong, 1993). The MP level was adjusted to 40 mg/mL as the target protein concentration using the Biuret method (Gornall *et al.*, 1949). Red bean protein isolate (RBPI, Saecharmdeul Co., Korea) were prepared as described by previous studies (Jang and Chin, 2011; Jang *et al.*, 2015). Microbial transglutaminase (MTG, 1% enzyme and 99% maltodextrin, 1 U/g activity) was provided by Ajinomoto Food Ingredients LLC (ACTIVA-TG, USA). MP mixtures with or without the addition of 1% RBPI were mixed with 1% MTG and adjusted at pH 5.75, 6.0, 6.25, or 6.5 using a food mixer (Bowl Rest™, Hamilton Beach/Proctor-Silex Inc, USA) as previously described (Lee and Chin, 2013). MP mixtures (5 g) were put into the glass vials, stored at 4°C refrigerator for 4 h for incubation, and then heated in a water bath (WB-22, Daihan Scientific Co., Korea) from 20 to 80°C to make the heat-induced protein gels as followed by Jang *et al.* (2015).

### Cooking yield

After cooking, heat-induced MP gels were chilled in an ice bath and kept in a refrigerator prior to measure cooking yield (CY, %) according to the following formula:

$$\text{Cooking yield (CY, \%)} = B / A \times 100$$

where A is the weight of uncooked MP and B is the weight of cooked MP.

### Gel strength

The MP sample was cooked in a water bath, and the gel strength was measured in the first peak value which was generated from breaking force (gf) during puncture tests, and expressed as gel strength (GS, gf) using the Merlin program of the Universal Testing Machine (3344, Instron,

USA) as described previously (Jang and Chin, 2011). A load cell of 500 N and cross speed of 50 mm/mm were used to measure the gel strength using puncture probe of 9 mm (diameter) to break the gel after the MP gel were equilibrated at room temperature (Lee and Chin, 2013).

#### Differential scanning calorimetry (DSC)

A DSC (S-650, Scinco Co., Korea) was used to evaluate the thermal denaturation of MP protein gels as affected by the addition of 1% MTG with or without 1% RBPI at pH 5.75, 6.0, 6.25, and 6.5. A calibration was performed with indium as a standard and an empty aluminum pan was used as a reference. Approximately 15 mg of sample mixtures were applied to measure the denaturation temperature ( $T_d$ ) by heating from 25 to 95°C in increments of 10°C/min as described previously (Lee and Chin, 2013).

#### Scanning electron microscopy (SEM)

The microstructure of the heat-induced MP gels were evaluated to investigate the three-dimensional changes using a scanning electron microscope (SEM) (JSM-7500F, JEOL Ltd., Japan) as described previously (Lee and Chin, 2013). To prepare the fixation, heat-induced MP gel samples (27 mm<sup>3</sup>) were soaked in buffer (0.1 M sodium phosphate and glutaraldehyde, pH 7.0) at 4±1°C for 24 h, and post-fixed in 1% osmium tetroxide (OsO<sub>4</sub>). The dehydration of post-fixed samples was performed with ethanol (50, 60, 70, 80, 90, and 3 times of 100%) by increasing concentrations for 10 min in each step as described by Haga and Ohashi (1984). Acetone was also applied to finalize full dehydration of the samples for 10 min (Hong and

Chin, 2010). To measure the microstructure, dehydrated heat-induced MP samples were gold-coated and then examined using a SEM analyzer at 15 kV and a magnification of × 1000.

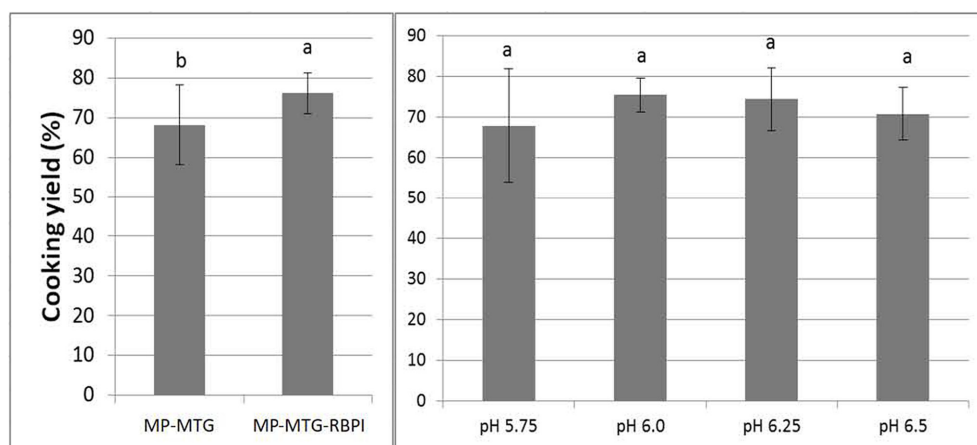
#### Statistical analysis

The result of replicate experiments (n=3) were statistically analyzed by using the SPSS (Program v. 20.0, SPSS, USA) with two-way ANOVA. When interaction between factors (pH values and RBPI treatment) was not significant, data were pooled by each factor or separated out when significant. When significant differences at  $p < 0.05$  were found, *post hoc* analysis was performed to compare the means by Duncan's multiple range tests (Lee and Chin, 2013).

## Results and Discussions

#### Cooking yield (CY, %)

Fig. 1 shows the CY results of the heat-induced MP gels mediated by 1% MTG with or without 1% RBPI at various pH values (5.75, 6.0, 6.25, and 6.5). Since the interaction between 1% RBPI and pH was not significant ( $p > 0.05$ ), the mean CY values were pooled. The addition of 1% RBPI improved the CY of the MP gels, regardless of pH values (5.5-6.5). When 1% RBPI was combined with MP mediated by MTG, it might be considered as a MTG substrate and water binder with potential to obtain proper protein gel formation and functionality. This result indicated that the addition of 1% RBPI increased the CY of MP gels by mitigating the negative effects of MTG addition on the water holding during cooking by accelerating

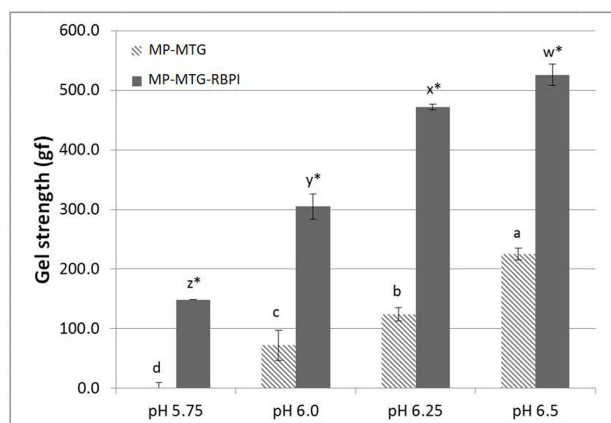


**Fig. 1.** Cooking yield (%) of porcine myofibrillar protein (MP) gels mediated by microbial transglutaminase (MTG) as combined with red bean protein isolates (RBPI) at various pHs. <sup>a,b</sup>Means with same superscript in different treatments and pH levels are not different ( $p > 0.05$ ).

protein-protein interaction as a squeezing effect (Jang *et al.*, 2015). Hong *et al.* (2012) reported that the CY of emulsified gels of pork myofibrillar protein mediated by MTG and combined with calcium alginate at various pHs increased at pHs higher than 6.0 or lower than 5.5. Hong and Xiong (2012) also reported that changes in protein structure were dependent on protein solubility when the pH was lowered to 3.0 as compared to pH 5.0. Approximately 42% changes in structure reflected the relatively greater protein solubility at the lower pH due to the increased protein amino groups. Similarly, Ke and Hultin (2005) reported that the protein gel functionality and rheological properties of protein-protein interactions of chicken breast meat in a low salt condition increased at pH 3.16, as compared to pH 3.70 or 4.06. These observations indicated that the low pH condition promotes gel formation at certain conditions. However, CY of MP mediated by MTG alone or combined with RBPI was not significantly different as affected by pH values (5.75-6.5) in this study. This result indicated that multiple combination may compromise to the effects of pH on the water holding capacity of MP gels mediated by MTG alone or combine with RBPI.

### Gel strength (GS, gf)

GS results of heat-induced protein gels mediated by 1% MTG with or without 1% RBPI at various pH conditions are shown in Fig. 2. GS increased when 1% RBPI was added, regardless of pH values (5.75-6.5) ( $p < 0.05$ ). This reflects the capability of RBPI to be a substrate in the MTG-mediated condition as well as the marked protein solubility of RBPI in a wide range of pH values (Meng and Ma, 2002b). GS also increased due to the increased hydrophobic and ionic bonding with MP by increased pH, regardless of the addition of RBPI ( $p < 0.05$ ), which reflects the increase of carboxyl groups. Hong and Xiong (2012) reported that the changes of a gel-forming protein were dependent on the heating temperature and the pH, being with pH 6 yielding the hardest gels. Lesiow and Xiong (2003) reported that pH 6.3 was optimum for gel properties of chicken breast meat. In this study, the hardest gels formed at pH 6.5 (Fig. 2), because electrostatic interactions between the MTG substrate and carboxyl groups of protein are formed, resulting in the increased protein-protein interactions (Jang *et al.*, 2015) when 1% RBPI was added. Thus, the maximum gel strength of MP mixed gel might be affected by the conditions of several other factors, such as heating temperature and ionic strength of the mixture.



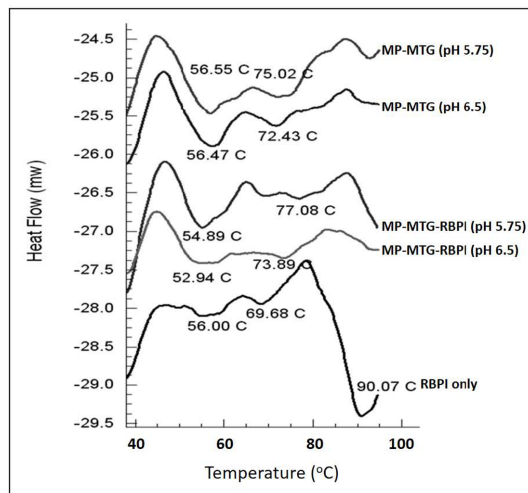
**Fig. 2.** Gel strength (gf) of porcine myofibrillar protein (MP) gels mediated by microbial transglutaminase (MTG) as combined with red bean protein isolates (RBPI) at various pHs. <sup>a-d</sup>Means with same superscript in a same treatment (MP-TG) are not different ( $p > 0.05$ ). <sup>w-z</sup>Means with same superscript in a same treatment (MP-TG+RBPI) are not different ( $p > 0.05$ ). \*Means with \* symbol in a MP-MTG with or without RBPI are different ( $p > 0.05$ ).

### Thermal Analysis

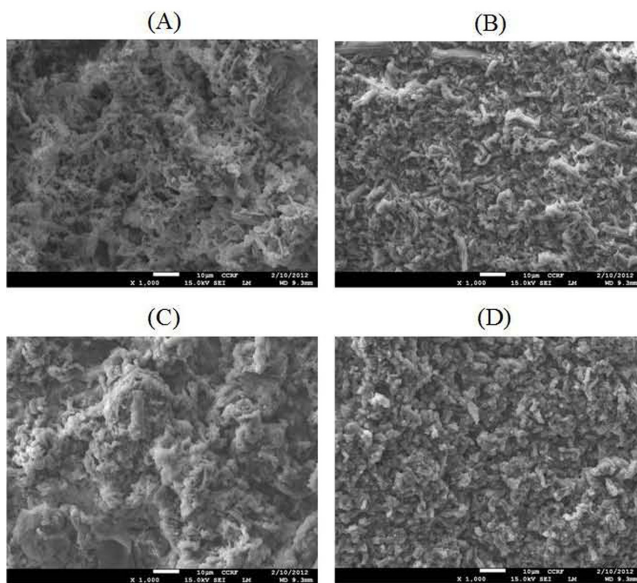
Thermograms of MP mediated by 1% MTG with or without 1% RBPI at pH 5.75 and 6.5 are shown in Fig. 3. Three endothermic peaks were generally observed, usually in MP samples. Deng *et al.* (2002) reported that the endothermic peaks of pork meat protein appeared at 52-54°C, 62-64°C, and 75-77°C. Hong and Chin (2010) also reported that the peak temperature of MHC, myosin light chain (MLC), and actin was 58, 66, and 75°C, respectively. These results indicated that endothermic peak temperatures could be slightly changed, due to environmental conditions, such as different muscle type, pH, and temperature. In this study, the addition of 1% RBPI at the same pH slightly reduced the first peak, which corresponds to the denaturation temperature of MHC. Shang and Xiong (2010) reported that rearrangements of protein structures in combined with MTG and 1% RBPI might represent increased enthalpy due to the formation of cross-links and hydrogen bonds. The second peak temperature, at denatured MLC, was increase by 2°C at the same pH. These results were partially due to the cross-linking of catalyzed MTG in MP with RBPI increases gel stability during heating (Damodaran and Agyare, 2013).

### Microstructure

Microstructures of heat-induced porcine MP gels induced by MTG and 1% RBPI at pH 5.75 and 6.5 are shown in Fig. 4. The three-dimensional structure of MP media-



**Fig. 3.** Thermograms of porcine myofibrillar protein (MP) gels mediated by microbial transglutaminase (MTG) as combined with red bean protein isolates (RBPI) at pH 5.75 and 6.5. MP, myofibrillar proteins; MTG, microbial transglutaminase; RBPI, red bean protein isolates.



**Fig. 4.** Microstructures of porcine myofibrillar protein (MP) gels mediated by microbial transglutaminase (MTG) (MP) as combined with or without red bean protein isolates (RBPI) at pH 5.75 and 6.5. (A) MP only (pH 5.75), (B) MP only (pH 6.5), (C) MP+RBPI (pH 5.75), (D) MP+RBPI (pH 6.5). Magnification  $\times 1,000$ .

ted by MTG was solid and compact when pH was increased, regardless of RBPI addition. This was partially due to the weak electrostatic binding of myosin that formed the void structure at pH 5.75. When the binding of proteins occurs rapidly, the gel network will be rough and

have a low water holding capacity (Liu *et al.*, 2008), and the three-dimensional gel structure was most suitable at pH 6.25. Thus, the addition of 1% RBPI increased the density of the gel structure with swelling markedly when the RBPI as a MTG substrate was present in MP gels. In addition, strong gels were formed by increases of the protein solubility and water holding capacity when 1% RBPI was added (Fig. 2). Furthermore, the three-dimensional structure was highly dense and more compact at pH 6.5 than those at pH 5.75 when RBPI was added.

## Conclusions

CY and GS of heat-induced porcine MP gels mediated by MTG were increased with the addition of 1% RBPI at increased pH values. The addition of RBPI with increased pH decreased the first endothermic peak temperature and became more compact with fewer voids at a three-dimensional structure. Heat-induced MP gels (0.3 M salt) mediated by MTG with 1% RBPI as a meat and water binder at pH 6.5 was selected as an optimum condition to improve the protein gel functionality. For further study, the optimum conditions of this study can be applied and evaluated in the scaled-up meat product.

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