

## Effects of Red Bean (*Vigna angularis*) Protein Isolates on Rheological Properties of Microbial Transglutaminase Mediated Pork Myofibrillar Protein Gels as Affected by Fractioning and Preheat Treatment

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### Abstract

Fractioning and/or preheating treatment on the rheological properties of myofibrillar protein (MP) gels induced by microbial transglutaminase (MTG) has been reported that they may improve the functional properties. However, the optimum condition was varied depending on the experimental factors. This study was to evaluate the effect of red bean protein isolate (RBPI) on the rheological properties of MP gels mediated by MTG as affected by modifications (fractioning: 7S-globulin of RBPI and/or preheat treatment (pre-heating; 95°C/30 min): pre-heating RBPI or pre-heating/7S-globulin). Cooking yields (CY, %) of MP gels was increased with RBPI ( $p < 0.05$ ), while 7S-globulin decreased the effect of RBPI ( $p < 0.05$ ); however, preheating treatments did not affect the CY ( $p > 0.05$ ). Gel strength of MP was decreased when RBPI or 7S-globulin added, while preheat treatments compensated for the negative effects of those in MP. This effect was entirely reversed by MTG treatment. Although the major band of RBPI disappeared, the preheated 7S globulin band was remained. In scanning electron microscopic (SEM) technique, the appearance of more cross-linked structures were observed when RBPI was prepared with preheating at 95°C to improve the protein-protein interaction during gel setting of MP mixtures. Thus, the effects of RBPI and 7S-globulin as a substrate, and water and meat binder for MTG-mediated MP gels were confirmed to improve the rheological properties. However, preheat treatment of RBPI should be optimized.

**Keywords:** red bean protein isolates, 7S-globulin, preheating, MTG-mediated myofibrillar protein gels, rheological properties

Received July 14, 2016; Revised September 27, 2016; Accepted September 30, 2016

### Introduction

Legume seed proteins have been generally used as non-meat ingredients to improve the water holding capacity and water binding ability of meat products, as well as to boost nutrition (Asghar *et al.*, 2010). Several studies have focused on the 7S globulin of soy, red bean, and mung-bean to evaluate the functional and structural interactions between food proteins. Physicochemical, functional, and structural characteristics of these proteins and their 7S globulins were investigated by evaluating the heat stability, protein solubility, pH, surface hydrophobicity, emulsion stability, and secondary structures (Tang and Sun, 2011). Mundi and Aluko (2012) extracted globulins from

kidney bean protein fractions and reported higher gelling properties and emulsion stability than albumin. This is partially due to the increased surface hydrophobicity of globulin, which might increase the gelling properties induced by protein-protein interactions.

Red bean (*Vigna angularis*, RB; adzuki bean or pat) protein consisted of 7S and 11S globulins, and contains large amounts of essential amino acids (Chau and Cheung, 1998). Red bean protein isolate (RBPI) has been suggested as a useful substrate for improving the water binding ability and textural properties, as well as emulsion stability in different addition levels, pH values, and salt concentrations to optimize processing conditions (Jang and Chin, 2011) when incorporated into myofibrillar protein (MP) gels mediated by microbial transglutaminase (MTG). Because MP gels can be affected by processing conditions including pH, salt concentration, temperature, and functional ingredients, their optimized application has attracted research interest (Chin *et al.*, 2009a; Chin *et al.*,

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2009b; Doerscher *et al.*, 2003; Kerry *et al.*, 1999; Ramirez-Suarez and Xiong, 2003).

Although there have been many studies on the functionality of legume seed proteins, the effects of fractioning (7S-globulin of RBPI) and/or preheating treatment (pre-heating; 95°C/30 min) on the rheological properties of MP gels induced by MTG are unclear. Thus, the objective of this study was to evaluate the effects of RBPI on the rheological properties of MTG-mediated MP gels as affected by fractioning and/or preheat treatment.

### Materials and Methods

#### Preparation of MP isolates and nonmeat ingredients

Approximately 200 g of pork loin meat (crossbred pigs: Landrace × Large Yorkshire) × Duroc; grade A, 110 kg live weight; Korea) was prepared as cubes (1-2 cm<sup>3</sup>), which were trimmed of excess fat and connective tissues. The cubes were vacuum-packed and frozen at -20°C until used. MP was prepared from pork loin cuts by homogenization and extraction with buffer solutions (0.1 M NaCl and 50 mM NaH<sub>2</sub>PO<sub>4</sub>; pH 6.25), and then finally obtained after centrifugation (J2-21, Beckman Inc., USA) at 1000 ×g for 15 min (Xiong, 1993). MP concentration was adjusted to 40 mg/mL using the Biuret method (Gornall *et al.*, 1949). RBPI was prepared from red bean (Saecharmdeul Co., Korea) according to the previous study (Jang and Chin, 2011; Kim *et al.*, 1990). RBPI 7S globulin protein was prepared by fractioning (Hong *et al.*, 2012) and/or preheating at 95°C for 30 min (pre-heating RBPI and pre-heating/7S-globulin) (Fig. 1). For MTG (ACTIVA TG-TI, 1% enzyme and 99% maltodextrin, 1 U/g activity; USA)-mediated MP gels, 1% of MTG was added to MP samples with 4 h incubation (Sakamoto *et al.*, 1994).

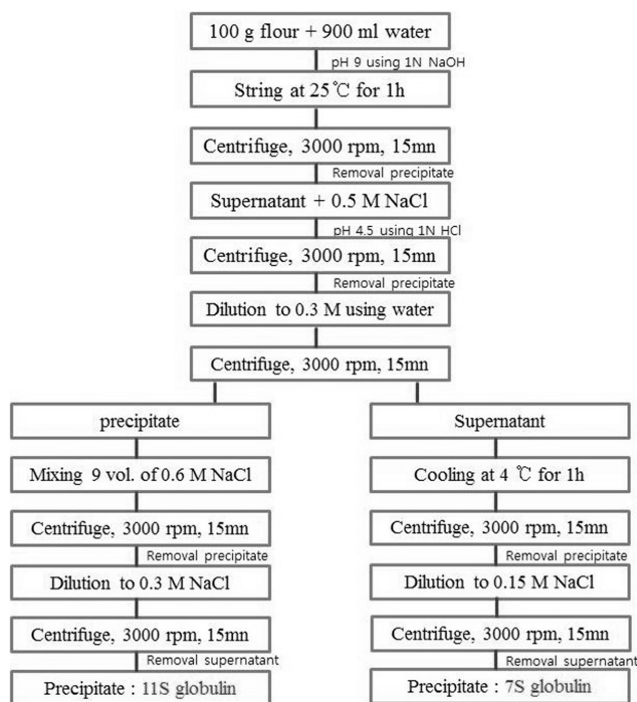


Fig. 1. Flow chart of red bean protein isolates and 7S-globulin preparation procedures.

#### Heat-induced protein gels

MP (0.45 M salt, pH 6.25) was admixed with/without MTG (1%) and preheat treatment of RBPI in a Bowl Rest™ food mixer (Hamilton Beach/Proctor-Silex, Inc., USA) (Table 1). MP mixtures (5 g) were loaded into glass vials, and refrigerated for 4 h to react with MTG (Sakamoto *et al.*, 1994). Heat-induced gels were prepared by cooking with increasing temperatures from 20 to 80°C at a rate of 3°C/min. After chilling the heat-induced MP gels in an ice/water, the gels were stored at 4°C until analyzed within 2 d.

Table 1. The formulation for the preparation of pork myofibrillar protein mixtures

Ingredients	Myofibrillar protein isolates (MP; mg/mL)									
	No MTG					1% MTG				
	No RBPI	RBPI	7S-globulin	pre-heating RBPI	pre-heating/7S-globulin	No RBPI	RBPI	7S-globulin	pre-heating RBPI	pre-heating/7S-globulin
1) MP	40	40	40	40	40	40	40	40	40	40
2) MTG	-	-	-	-	-	10	10	10	10	10
3) RBPI	-	10	-	-	-	-	10	-	-	-
4) 7S-RBPI	-	-	10	-	-	-	-	10	-	-
5) Pre-HT/RBPI	-	-	-	10	-	-	-	-	10	-
6) Pre-HT/7S-RBPI	-	-	-	-	10	-	-	-	-	10

Treatments: pork myofibrillar protein (MP) gels mediated by microbial transglutaminase (1% MTG) with or without 1% red bean protein isolate (1% RBPI) as affected by fractioning (7S-globulin) and/or preheating treatments (pre-heating RBPI or pre-heating/7S-globulin).

## Analyses of MP gel property

### Cooking yield and gel strength

After heating, cooking yield (CY, %) and gel strength (GS, gf) of heat-induced MP gels were evaluated. CY was measured by weight differences between before and after cooking as:

$$\text{Cooking loss (CL, \%)} = (A - B) / A \times 100$$

$$\text{Cooking yields (CY, \%)} = 100 - \text{CL}$$

A: weight of uncooked MP; B: weight of cooked MP.

GS was measured by compression test using an Instron Universal Testing Machine and the Merlin program (Instron, USA) with a 500 N load cell at 50 mm/min cross speed. The samples were equilibrated to room temperature for the puncture test using a 9 mm diameter puncture probe. When the heat-induced, chilled, and equilibrated MP gels were ruptured after compression test, the first peak value of breaking force (gf) was determined as the GS (Lee and Chin, 2013).

### Sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE)

As shown in Table 1, MP mixtures prepared with RBPI and 7S-globulin, and preheating were used for SDS-PAGE using a Mini-PROTEAN 3 Cell system (Bio-Rad Laboratories, USA) with a 10% separating and a 4% stacking gels (0.375 M Tris, pH 8.8 and 0.125 M Tris, pH 6.8, respectively) (Laemmli, 1970). Electrophoresis was run at 150 V for 1.5 h with raw extracted protein samples diluted by sample buffer (4% SDS, 20% glycerol, 20% mercaptoethanol, and 0.125 M Tris, pH 6.8) to load the protein (1%,  $\mu\text{g}/\mu\text{L}$ ) per each well. After run, the gels were dyed using Commassie Brilliant Blue R-250 (Bio-Rad Laboratories, Hercules, USA) during shaking for 30 min and then, de-stained 3 times with 1 h interval using a model RK-120 rocker (New Power Co., Korea). Standard marker consisting of pre-stained SDS-PAGE standards (Bio-Rad) was used to calculate the molecular weight of bands induced from each samples.

### Scanning electron microscopy (SEM)

SEM was performed to observe the differences of three-dimensional structure among treatments using a JSM-7500F microscope (JEOL Ltd., Japan) as previously described (Jang and Chin, 2011). According to prior methods

for sample preparation (Haga and Ohashi, 1984), cubic samples (3 mm<sup>3</sup>) were fixed by soaking for 24 h in 2.5% glutaraldehyde buffer solutions (0.1 M sodium phosphate buffer with glutaraldehyde, pH 7.0) at  $4\pm 1^\circ\text{C}$ . A post-fixation procedure was followed with osmium tetroxide (OsO<sub>4</sub>) buffer solution. Post-fixed samples were washed and dehydrated by dipping in increased levels of ethanol (50, 60, 70, 80, 90%, and three times with 100%) and acetone for 10 min per each step (Jang and Chin, 2011). To observe the microstructure (magnification,  $\times 2000$ ), samples were gold-coated and then measured using a SEM analyzer at 15 kV.

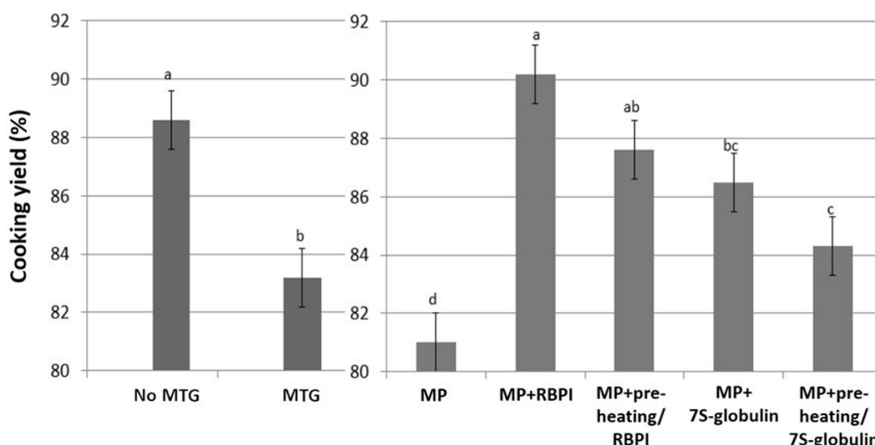
### Statistical analyses

All experiments were performed in triplicate ( $n=3$ ). SPSS version 20.0 (SPSS, USA) was used to analyze the statistical differences among treatments by Two-way ANOVA (Lee and Chin, 2013). Post-hoc testing was performed to compare the means using Duncan's multiple range test when statistical significances were found at  $p<0.05$ .

## Results and Discussion

### Cooking yield (CY, %) and gel strength (GS, gf)

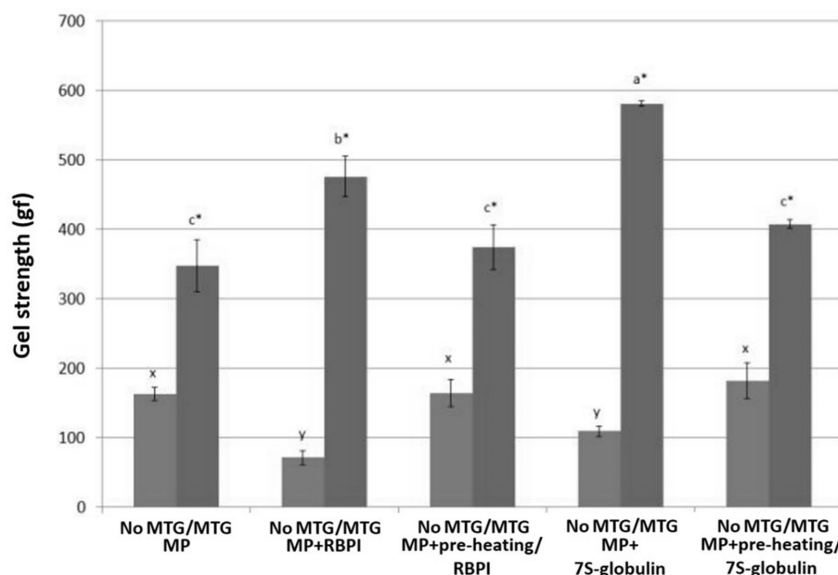
CY of MP gels was decreased with the addition of 1% MTG ( $p<0.05$ ; Fig. 2). The addition of MTG decreases CY due to the MTG catalyzed protein-protein interactions rather than protein-water interaction (Hong and Chin, 2010). However, presently the addition of 1% RBPI improved the CY of the MP gels, while fractioning (7S-globulin) decreased the effects of RBPI on the CY of MP gels ( $p<0.05$ ; Fig. 2); however, preheat treatment ( $95^\circ\text{C}/30$  min) did not affect the CY of MP gels ( $p>0.05$ ; Fig. 2). These results indicated that the water-soluble proteins of RBPI may affect the increased protein-water interaction, resulting in the improving CY, while the preheating procedure did not improve the cooking yield, since the hydrophobic interaction induced by preheating did not significantly change the CY of MP gels (Fig. 2). Hong-sprabhas and Barbut (1999) reported that the addition of whey protein prepared with preheat treatment increases the water holding capacity of cold-set gels and reduces the cooking loss of poultry meat products. Thus, pre-heating may or may not be advantageous for the water holding capacity of processed meat during heating, depending on the conditions. The increased temperature or heating time might be improved the water binding capacity during heating or storage.



**Fig. 2. Pooled means of the cooking yield (%) of myofibrillar protein (MP) gels.** Factors: microbial transglutaminase (MTG) treatment (MP with or without 1% MTG, MTG or No MTG) and red bean protein isolate (RBPI) addition with or without modifications (fractioning: 7S-globulin and/or preheat treatments, pre-heating: MP alone, MP+1% RBPI, MP+1% pre-heating/RBPI, MP+1% 7S-globulin, MP+1% pre-heating/7S-globulin); <sup>a,b</sup>Means with the same superscripts in the MTG treatment are not different ( $p>0.05$ ); <sup>a-d</sup>Means with the same superscripts in the RBPI addition are not different ( $p>0.05$ ).

For the gel strength (GS, gf), RBPI addition may negatively affect the GS of MP without 1% MTG, while RBPI increased the GS of MTG-mediated MP (Fig. 3;  $p<0.05$ ). Although RBPI was used as a substrate for MTG-mediated MP gels by increasing the protein-protein interactions, the hydrophilic fraction of RBPI may negatively affect the gelling property of MP alone. Sun and Arntfield

(2012) reported that MP with 1% pea protein mixtures induced by MTG has a higher GS than MP alone. Vicilin-rich protein isolates with MTG improves crosslinks between inter- and intra-molecular interactions (Tang, 2008). In the same manner, pre-heating 7S treatment increased the GS of MP induced by MTG; however, pre-heating treatments decreased the GS of MTG-mediated MP with



**Fig. 3. Effect of red bean protein isolate (RBPI) with or without modifications (fractioning: 7S-globulin and/or pre-heat treatments, pre-heating: pre-heating RBPI or pre-heating/7S-globulin) on the gel strength (gf) of myofibrillar protein (MP) gels mediated by 1% MTG or not (MTG or No MTG).** <sup>x,y</sup>Means with the same superscripts in the same treatment (MP) are not different ( $p>0.05$ ); <sup>a-c</sup>Means with the same superscripts in the same treatment (MP+MTG, MT) are not different ( $p>0.05$ ); \*Means with an asterisk (\*) are different (MP alone, MP versus MP+MTG, MT) in same treatment (No RBPI, 1% RBPI, 1% pre-heating/RBPI, 1% 7S-globulin, 1% pre-HT/7S-globulin) ( $p<0.05$ ).

RBPI or 7S-globulin (Fig. 3;  $p < 0.05$ ), even though MTG-mediated MP control was similar to preheated treatments (pre-heating RBPI or pre-heating/7S-globulin) (Fig. 3;  $p > 0.05$ ). These results indicated that the protein-protein interactions and hydrophobic interactions induced by folding proteins during gel setting process might improve gelling properties; however, the changes of RBPI residues induced by preheat treatment might affect the GS in MTG-mediated MP. Chanarat and Benjakul (2013) reported that MTG increases the GS of fish meat products; however, the addition of formaldehyde negatively affected the cross-links induced by MTG. Since the formaldehyde decreased the amounts of amino groups, the cross-links between protein residues catalyzed by MTG were decreased, while the fractioning might contribute the increased protein-protein interactions of MP gels, regardless of MTG treatment (Chanarat and Benjakul, 2013).

The addition of 7S showed the highest GS with addition of MTG-mediated MP mixtures. These results indicated that 7S-globulin could contribute to the increased protein-protein interaction when MP was catalyzed by MTG during gelling process. Tang (2008) reported that 7S-globulin had two different endothermic denaturation temperatures (87.7 and 94.1°C) and that disulfide bonds did not contribute to increase the gelling property; however, hydrophobic and hydrogen bonds mainly affected the gelation. Mundi and Aluko (2012) reported that globulin of kidney bean had higher gel forming ability and emulsion stability than albumin. The exposures of hydrophobic residues induced by heating might increase the protein-protein interactions in globulin of kidney beans and increased the gel forming ability. However, legume seed proteins generally decreased the textural properties of salt-soluble protein gels (Sun *et al.*, 2012). Based on the results, chemical modifications of RBPI should be done to optimize the gelling property of MP mediated by MTG. However, the preheating procedure decreased the gel strength of both MP with RBPI or 7S-globulin.

### SDS-PAGE profiles

The effects of RBPI on the SDS-PAGE profiles of MP with or without 1% MTG as affected by fractioning and modifications are shown in Fig. 4. RBPI can be divided into acidic (38-45 kDa) and basic subunits (11-24 kDa) having 7S vicilin and 11S legumin, respectively. The major bands of 7S vicilin was clearly found around at 38-45 kDa (Fig. 4A). Mundi and Aluko (2013) reported that the vicilin of kidney beans was found at 43 or 45 kDa in SDS-PAGE profiles. The major band of 7S-globulins was con-

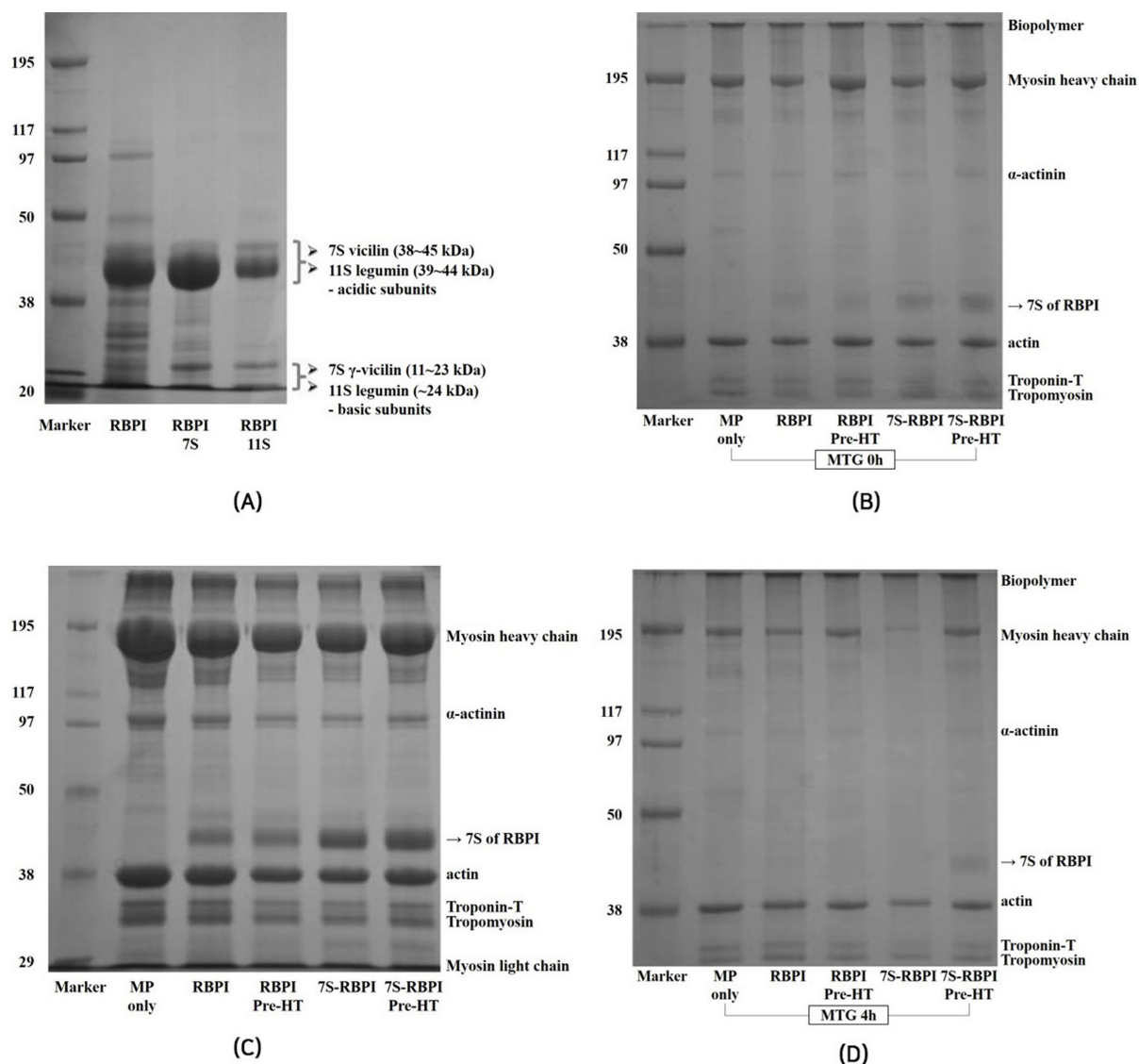
densed as compared to that of RBPI, regardless of preheat treatment in MP (Fig. 4B). Without incubation, MHC and major band of 7S-globulin clearly remained and there was no polymerisation to form biopolymer on the stacking gel, which could be formed by interactions between MP and functional proteins catalyzed by MTG treatment (Fig. 4C). MHC disappeared when 7S-globulin was added to MTG-mediated MP, while MHC of other treatments remained; however, the major band of 7S-globulin was observed when it prepared with preheat treatment (Fig. 4D). These results indicated that interactions between 7S-globulin and MTG-mediated MP gels was highly activated, resulting in highest GS; however, preheating might diminish the effects of 7S-globulin, resulting in lower GS of MTG-mediated MP gels.

### Microstructure

Microstructures of heat-induced MP gels with or without 1% MTG treatment as affected by the addition of 1% RBPI and modification are shown in Figs. 5 and 6, respectively. When RBPI was incorporated into MP gels, the microstructure became more compact regardless of modifications (fractioning and/or preheating treatments) of RBPI (Fig. 5). These results indicated that the modification of RBPI might change the role to form a gel structure of MP. Sun *et al.* (2012) confirmed the strengthened gel structures by the addition of peanut proteins in chicken salt-soluble protein gels. When both RBPI and 7S-globulin were incorporated into the MTG-mediated MP gels, void structures became filled and more uniform, and flat surface structures formed; however, preheating changed the surface structure and broke the strongly flat structures of MTG-mediated MP gel prepared with RBPI or 7S-globulin by changing the active sites of proteins (Fig. 6). Meng and Ma (2002) also reported that red bean globulin protein was changed by different heating temperatures between 90 and 95°C. When the preheating temperature was close to the endothermic denaturation temperature of 95°C, the gel structure became more compact and a strongly cross-linked structure was observed (Meng and Ma, 2002). This result also supported the appearance of more cross-linked structures were observed when RBPI was prepared with preheating at 95°C to improve the protein-protein interaction during gel setting of MP mixtures.

### Conclusions

RBPI and 7S-globulin (fractioning) improved the GS of MTG-mediated MP, while RBPI improved the CY, regard-



**Fig. 4.** SDS-PAGE profiles of red bean protein isolates (RBPI) and myofibrillar protein isolates (MP) as affected by MTG treatment and RBPI addition with or without modifications (fractioning: 7S-globulin and/or pre-heating, pre-heating: pre-heating/RBPI or pre-heating/7S-globulin). (a) RBPI and their fractions (7S and 11S globulins), (b) MP with or without 1% RBPI (MP+RBPI) as affected by fractioning (MP+7S-globulin) and/or preheating (MP+pre-heating/RBPI or MP+pre-heating/7S-globulin), (c) MP reacted with MTG (MP+MTG) at 0 h with RBPI (MP+MTG+RBPI) as affected by fractioning (MP+MTG+7S-globulin) and/or preheating (MP+MTG+pre-heating/RBPI or MP+MTG+pre-heating/7S-globulin), (d) MP reacted with MTG (MP+MTG) at 4 h with RBPI (MP+MTG+RBPI) as affected by fractioning (MP+MTG+7S-globulin) and/or preheating (MP+MTG+pre-heating/RBPI or MP+MTG+pre-heating/7S-globulin)

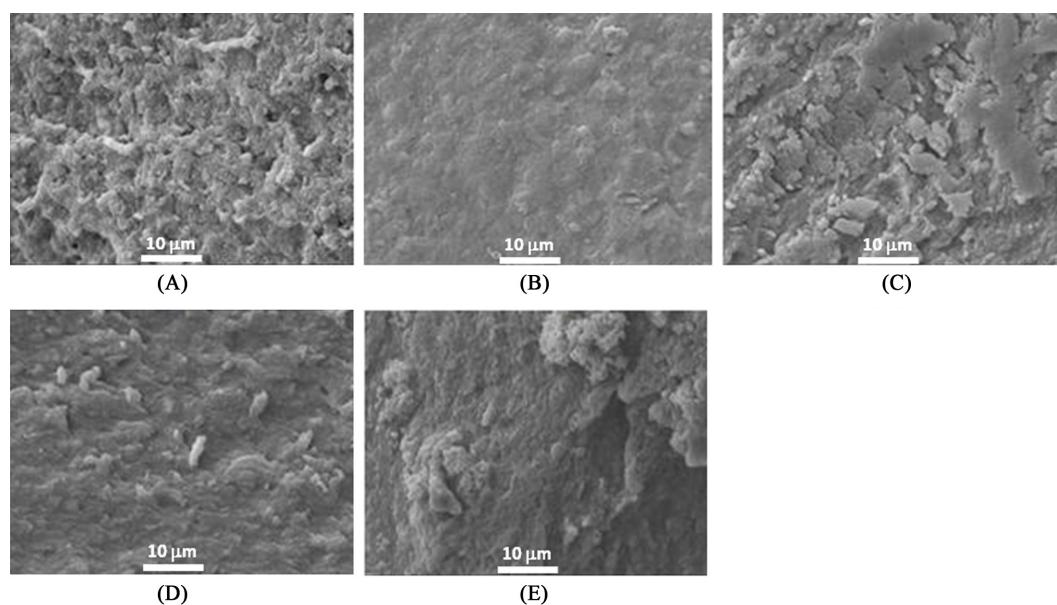
less of modifications. These results was confirmed by compacted surface structures in SEM, and major band disappearances of RBPI in MP gels. However, the stable surface structures of MTG-mediated MP gels were changed and broken by the addition of RBPI or 7S-globulin treated by pre-heating. Thus, RBPI and 7S-globulin are suggested to improve the rheological properties of MTG-mediated MP gels as a substrate for MTG to function as a water and meat binder.

## Acknowledgements

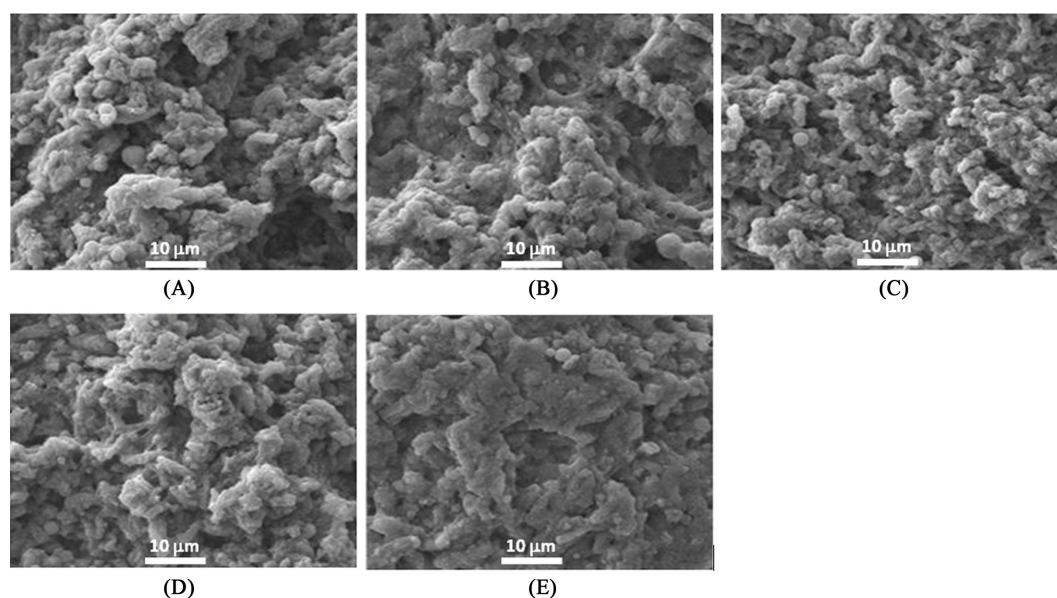
This study was financially supported by National Research Foundation (NRF Project # 2014 009279).

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**Fig. 5.** Microstructures of red bean protein isolates (RBPI) and myofibrillar protein isolates (MP) as affected by RBPI addition with or without modifications (fractioning: 7S-RBPI and/or preheating, preheating: pre-heating/RBPI or pre-heating/7S-globulin) (magnification,  $\times 2000$ ). (A) MP alone, (B) MP+RBPI, (C) MP+pre-heating/RBPI, (D) MP+7S-globulin, (E) MP+pre-heating/7S-globulin.



**Fig. 6.** Microstructures of red bean protein isolates (RBPI) and myofibrillar protein isolates (MP) as affected by MTG treatment and RBPI addition with or without modifications (fractioning: 7S-globulin and/or preheating, pre-heating: pre-heating/RBPI or preheating/7S-globulin) (magnification,  $\times 2000$ ). (A) MP+MTG, (B) MP+MTG+RBPI, (C) MP+MTG+pre heating/RBPI, (D) MP+MTG+7S-globulin, (E) MP+MTG+pre heating/7S-globulin.

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