EFFECT OF DIFFERENT LEVELS OF RED BEAN PROTEIN AND VARIOUS SALT LEVELS ON RHEOLOGICAL PROPERTIES OF TRANGLUTAMINASE-MEDIATED MYOFIBRILLAR PROTEIN GEL

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Abstract – The objective of this study was to investigate the effect of different levels of red bean protein (RBP) and various salt levels on rheological properties of microbial transglutaminase (MTG)-mediated pork myofibrillar protein gel. Cooking yield (%) increased with the addition of red bean protein regardless of level of RBP, and increased at 0.3 M or 0.45 M salt, as compared to 0.15 M. Both increased levels of RBP and increased salt level (>0.3 M salt) in MTG-mediated MP gels increased gel strength. The second peak of differential scanning calorimetry (DSC) increased with addition of red bean protein. The SDS-PAGE showed that myosin heavy chain disappeared with increased salt levels. Also, invisible red bean protein bands were occurred at 0.3 M or 0.45 M salt levels. Scanning electron microscopic techniques (SEM) showed more compact and less void with increased salt level or with the addition of red bean protein. There results indicated that there were some interactions between red bean protein and MTG in myofibrillar protein gel.

Key Words – red bean protein, transglutaminase, myofibrillar protein, salt levels

I. INTRODUCTION

Myofibrillar protein (MP) played an important role in meat processing by forming a viscoelastic texture, called heat-induced gel [1]. Functional properties of MP gels were gel strength, fat binding and water holding capacity [2]. There were many factors affecting functional properties of MP, such as ionic strength (salt concentrations), pH and other ingredients.

Salt added to the meat products to improve flavor, safety, texture characteristics and preservation [3]. Also, it has been widely used to manufacture the various meat products with heat treatment. However, because of the negative effects of sodium chloride in the implementation of hypertension or cancer, public health has recommended a reduced intake of salt for consumer. In addition, consumer required low salt level on meat and meat products. Thus, low salt meat system will continually carry out to maintain of meat quality and improve the rheological properties, such as hardness or elasticity on meat products [4]. For this reason, many studies conducted a research on replacement of sodium chloride with non-meat protein, hydrocolloid and enzyme [5,6].

Microbial transglutaminase (MTG) is an enzyme catalyzing the protein cross-linking through the covalent bond, results in gel strength, hardness and cohesiveness improvements [7]. MTGs have been widely used in meat processing in order to improve textural properties of meat products [8]. Red bean protein (RBP), which is one of the non-meat protein, it could be formed gel and RBP have a respectable amount lysine residue [9]. Therefore, RBP could be improving functional properties of MTG-mediated MP gel. The aim of this study was to evaluate the effect of various salt levels and RBP combinations on gelling properties of MTG-mediated MP gel.

II. MATERIALS AND METHODS

1. Preparation of myofibrillar protein and functional ingredients

Myofibrillar protein isolate (MPI) was prepared from pork loin by washing three times with 4 volumes (v/w) of 0.1 M NaCl, 50 mM NaH_2PO_4 buffer (pH 6.25), followed by washing with eight volume (v/w) of 0.1 M NaCl (pH 6.25) and adjusted to targeted pH 6.25 using 1 N HCl or NaOH (Hong and Chin, 2010) and centrifuged at 3000 x g at 4℃ for 15 min (Beckman, Palo Alto, CA, USA). Protein concentration was determined by the biuret method. Red bean protein was prepared from red bean flour by isoelectric precipitation method [5]. Microbial transglutaminase (MTG) was donated by Ajinomoto Food Ingredients (TG-as, South Korea).

2. Cooking yields (%) and gel strength (gf)

Cooking yield was determined after cooking in a water bath from 5 to 80℃. $(W_a/W_I)\times 100$; W_a is the weight of the remaining gels. W_I is the weight of the initial gels. A puncture test determine to the gel strength. The sample were using an Instron model (3340, Instron Corporation, Canton, MA, USA). The probe diameter was 9 mm and head speed was at 50 mm/min.

3. Differential scanning calorimetry (DSC)

Thermal analysis was determined the effect of red bean protein on the denaturation of MP mixed gel with MTG using by differential scanning calorimetry (DSC S-650, Scinco Co., South Korea). DSC was performed by 10℃/min heating rates from 25 to 90℃.

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

SDS-PAGE was determined to identify MP polymerization with MTG during 4h incubation (4℃). A 10% acrylamide separation gel and a 4% acrylamide stacking gel were used [10]. Electrophoresis was using a Mini-PROTEAN 3 cell apparatus (Bio-Rad Laboratories, CA, USA). The running condition was 150V of voltage for approximately half an hour. The gels were put into 1% dye solution (Commassie Brilliant Blue R-250, Bio-Rad, CA, USA). The gels were put into destaining solution in order to decolorize for 1h at three times. SDS-PAGE gels checked using photoshop program.

5. Scanning electron microscopy (SEM)

Scanning electron microscopy was performed using FE-SEM (JSM-7500F, JEOL Ltd., Tokyo, Japan). Samples were fixed using a 0.1 M sodium phosphate buffer containing 2.5% glutaraldehyde (pH 7.0) for 24h at 4℃. The fixed samples were for 5h in 1% osmium tetraoxide (pH 7.0). The samples washed three times with 0.1 M phosphate buffer (pH 7.0) for 10 min and then dehydrated in increasing concentrations of ethanol (50, 60, 70, 80, 90 and 100%) for 10 min per solution.

III. RESULTS AND DISCUSSION

1. Cooking yield (%) and gel strength (gf)

The results of cooking yield (CY, %) and gel properties of MTG-mediated myofibrillar protein gel with red bean protein (RBP) at various salt levels are shown in Tables 1 and 2. CYs increased with increased salt levels ($p<0.05$), however, they were not different between 0.3 M or 0.45 M salt levels ($p>0.05$). Also, CYs increased with the addition of RBP, regardless of RBP level. These results were partially due to inclusion of hydrophilic amino acids, such as aspartic acid or glutamic acid in RBP [9].

Table 1. Cooking yield (%) of myofibrillar protein gels treated with microbial transglutaminase (MTG) and red bean protein (RBP) at various salt levels

salt levels			red bean protein levels		
015 M	0.3 M	0.45 M	0 %	1 %	2 %
57.1 ± 8.6^{b}	72.0 ± 6.6^{a}	76.1 ± 5.9^{a}	62.2 ± 7.5^{b}	69.1 ± 10^{a}	74.0 ± 11^{a}

^{a,b} Means with same superscript in a same row are not different ($p>0.05$). MP: myofibrillar protein; RBP: red bean protein

Gel strength increased with the addition of RBP, except for 0.15 M salt level ($p<0.05$). Also, gel strength values were increased up to 0.3 M salt and no further increases were observed thereafter (>0.3M salt).

Table 2. Gel strength (gf) of myofibrillar protein gels treated with microbial transglutaminase (MTG) and red bean protein (RBP) at various salt levels

Treatments	Salt levels		
	0.15 M	0.3 M	0.45 M
MP	89.4 ± 26^{cA}	250 ± 31^{bC}	420 ± 51^{aC}
MP+RBPI(1%)	107 ± 37^{bA}	642 ± 13^{aB}	671 ± 114^{aB}
MP+RBPI(2%)	99.9 ± 22^{cA}	794 ± 45^{bA}	894 ± 48^{aA}

$^{A\text{-}C}$ Means with same superscript in a same column are not different ($p>0.05$).

$^{a\text{-}c}$ Means with same superscript in a same row are not different ($p>0.05$).

MP and RBP: see Table 1

Therefore, gel properties of MTG-mediated MP in the presence of RBP (1%) at 0.3 M salt were higher than those with 0.45 M salt level. Pietrasik et al. (2007) [6] reported that non-meat protein improved texture and water-holding capacity on meat system.

2. DSC

Thermal denaturation of myofibrillar protein has three distinctive endothermic peaks at approximately myosin heavy chain (60℃), myosin light chain (68℃), actin (74℃), respectively [11]. Overall, the first peaks of DSC were not changed, while the temperature of second peak increased with the addition of RBP. These results might be partially due to the changes of protein structure through cross-linking with the combination of RBP and MTG. Tang et al. (2006) [12] reported that the changes of thermal properties were caused through cross-linking with the combination of soy protein isolate and MTG.

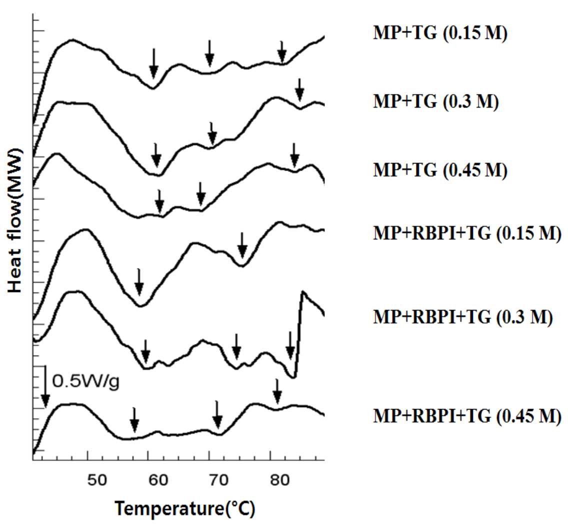

Figure. 1. Differential scanning calorimetry (DSC) of TG-mediated MP gel with or without RBP (2%) at various salt levels

3. SDS-PAGE

SDS-PAGE technique was performed to determine the changes of protein pattern with the addition of RBP and MTG (Fig. 2). Myosin heavy chain disappeared with increasing salt levels, and a biopolymer was observed in all treatments [13]. Also, SDS-PAGE results showed a protein band from RBP was observed, while no bands from RBP were observed at 0.3 M and 0.45 M. Therefore, RBP could be interaction with MTG more 0.3 M salt level.

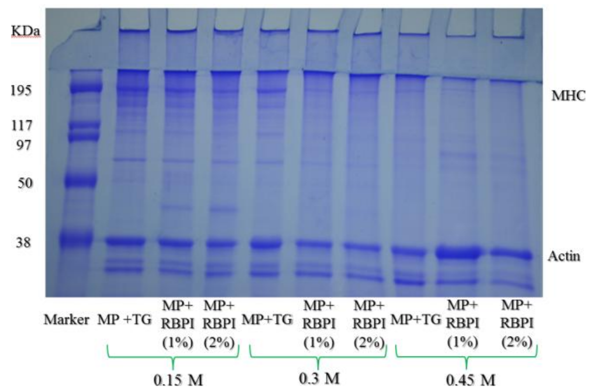

Figure. 2. SDS-PAGE pattern of TG-mediated MP gel with increased levels of RBP at various salt levels.

4. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) of myofibrillar protein with RBP and MTG are showed in Fig. 3. The structures of MP with RBP and MTG showed more compact than those with MP with MTG. Thus, RBP might be more interacted with MTG than myofibrillar protein did. Thus, the SEM data similarly indicated that

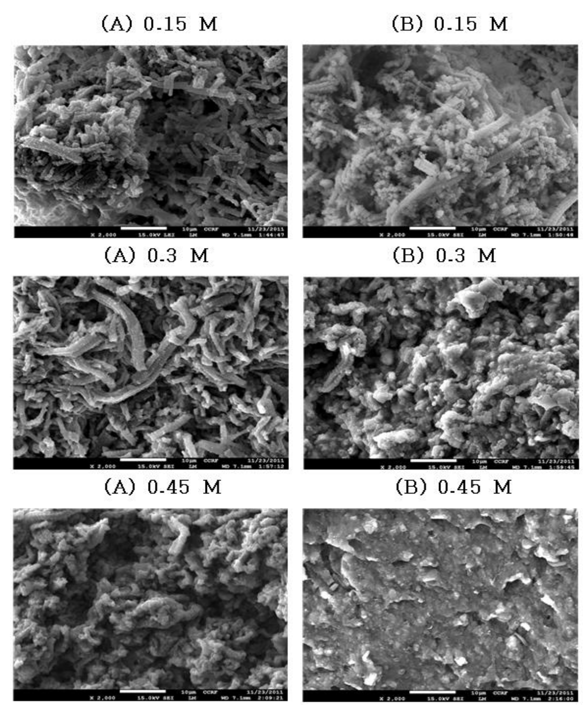

Figure. 3. Scanning electron microscopy (x2000) of TG-mediated MP gel with or without RBP at various salt levels. (A): myofibrillar protein; (B): myofibirillar protein + red bean protein (2%).

Cooking yield or gel strength increased with the addition of RBP (1~2%) at 0.3 M and 0.45 M salt levels. Also, the results of SEM showed that increased salt levels decreased void or space and increased swelling structure were observed [14]. These results were possibly due to the more protein solubility at higher salt levels, resulting in. in increasing cooking yield with the addition of RBP (Table 1).

IV. CONCLUSION

Cooking yield increased with the addition of red bean protein (RBP), regardless of RBP level and increased at 0.3 M or 0.45 M salt, as compared to 0.15 M. The increased level of RBP and salt increased gel strength, except for 0.15 M salt. The interactions between RBP and MTG in MP system were supported by DSC, SDS-PAGE and SEM results. Therefore, gel properties of MTG-induced MP with RBP (1%) at 0.3 M salt could be replaced with those at 0.45 M salt.

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