EVALUATION OF MUNGBEAN PROTEIN ISOLATE AS A SUBSTRATE FOR MICROBIAL TRANSGLUTAMINASE ON HEAT-INDUCED GELATION OF PORK MYOFIBRILLAR PROTEIN

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***Abstract*—this study was performed to evaluate the potential possibility of various levels (0, 0.27, and 0.53%) of mungbean protein isolate (MPI) as a substrate for transglutaminase (TG) interaction on the pork myofibrillar protein (MP). Heat-induced gel characteristics were evaluated by measuring cooking loss (CL), gel strength (GS)SDS-PAGE, thermograms, and microstructure. TG treatment increased CL and GS of mixed protein gels ($P<0.05$), while increased MPI level reduced CL. SDS-PAGE profiles of treatments with TG showed high molecular weight biopolymers, regardless of MPI level. However, the loss of a band of MPI alone at TG-mediated mixed protein gel indicated that MPI could be a potential substrate for TG. The MP control without TG and MPI (CTL) showed three endothermic peaks at approximately 55, 63, and 74°C, corresponding to myosin heavy chain, myosin light chain, and actin, respectively. The TG treatment resulted in reduced peak temperatures of myosin light chain and vanished endothermic peak of myosin heavy chain (denaturation), indicating the initial changes of protein gelation. The addition of MPI slightly changed to the endothermic peaks, as compared to those of CTL. In microstructures, TG treatment affected the formation of a finely stranded structured in MP gels, while MPI showed a conglomerated surface in TG-mediated MP gels. In conclusion, the roles of MPI could be identified as a water binder and a substrate for TG interaction.**

***Index Terms*—mungbean protein isolate, pork myofibrillar protein gel, transglutaminase**

I. INTRODUCTION

Myofibrillar protein gelation was formed as following process; extraction, denaturation, aggregation, and gel formation (Tarte & Amundson, 2006). Protein gel functionality in meat systems could be explained by the ability to bind and retain water which were affected by intrinsic and extrinsic factors such as protein sources or processing treatments (Sebranek, 2009). Among extrinsic factors, meat processing ingredients including salts, phosphates, alkalis, acids, oxidants, antioxidants, nonmuscle proteins, and enzymes were commonly used to promote muscle protein functionality. Especially, transglutaminase is a beneficial cross-linking enzyme as a binding agent for improving gelling properties of muscle protein (Xiong, 2009). As functional ingredients, legume and their proteins can be also used in meat processing (Boye, Zare, & Pletch, 2010). On the other hand, mungbean (*Vigna radiata* (L) Wilczek) was reported that its seeds had an antioxidant activity (Anwar, Latif, Przybylski, Sultana, & Ashraf, 2007) and its flour recently used as a water and meat binder in a meat system (Lee & Chin, 2009). Thus, this study was performed to evaluate the potential possibility of mungbean protein isolate (MPI) with various levels (0, 0.26, and 0.53%) as a potential substrate for transglutaminase (TG) on the pork ham myofibrillar protein (MP).

II. MATERIALS AND METHODS

Pork myofibrillar protein gels (MP) were prepared by the procedure of Chin, Go, & Xiong (2009a) with several washing steps to obtain myofibrillar protein isolates (0.1 M NaCl and 50 mM NaH_2PO_4 buffer solutions; pH 6.25) by centrifugation at 1000 x g for 15 min. The concentration of myofibrillar protein was determined by the Biuret method (Gornall, Bardawill, & David, 1949). Then, heat-induced gels were prepared by increasing the internal temperature from 20 to 75°C at 3°C/min increments. Mungbean protein isolate (MPI) was prepared from mungbean flour (Hamyang Nong-Hyup, Hamyang, South Korea) by isoelectric precipitation method (Thompson, 1977). Microbial transglutaminase (TG; 1% TG with 99% maltodextrin) was donated by Ajinomoto Food Ingredients (ACTIVA TG-TI, Chicago, IL, USA). Table 1 shows the formulation for the preparation of pork myofibrillar protein gels.

Table 1. The formulation for the preparation of pork myofibrillar protein gels (μg/μL)

Compositions	NT			TG		
	CTL	T1	T2	CTL	T1	T2
MP	40	40	40	40	40	40
TG	10	10	10	10	10	10
MPI	0	2.67	5.33	0	2.67	5.33

Treatments: pork myofibrillar protein gel (MP) control (CTL) with 1% transglutaminase (TG) or without (NT); MP with 0.26% mungbean protein isolate (MPI) (T1); MP with 0.53% MPI (T2).

To evaluate the pork gel functionality, pH, cooking loss (%), gel strength (gf), sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE), differential scanning calorimetry (DSC), and scanning electron microscopy (SEM) were measured. Data were analyzed by PASW Statistics 18 (SPSS Inc., Chicago IL, USA) program.

III. RESULTS AND DISCUSSION

No differences in pH values of pork myofibrillar protein (MP) gel were observed as affected by transglutaminase (TG) treatment or different mungbean protein isolate (MPI) levels (P>0.05; Table 2). However, TG treatment increased cooking loss and gel strength of pork myofibrillar protein (MP) gel, while increased MPI level did not affect gel strength, but reduced cooking loss of MP gel (P < 0.05; Table 2). These results indicated that 1% TG addition improved gel strength and 0.53% MPI alone could be used as a water binder in MP gel system. Previous study reported that TG treatment greatly enhanced gel strength and elasticity at a higher salt level (0.6 M), while *konjac* flour (KF) improved cooking yield at all salt levels (Chin, Go, & Xiong, 2009a). They also reported that the combination of KF and TG improved the gel strength of heat-induced gel of porcine myofibrillar protein at low salt levels (0.1 and 0.3 M).

Table 2. Pooled means of pork ham myofibrillar protein gel functionality

Parameters	TG treatment		MPI level		
	NT	TG	0%	0.27%	0.53%
pH values	6.79 [a]	6.80 [a]	6.79 [a]	6.79 [a]	6.80 [a]
Cooking loss (%)	11.9 [b]	41.1 [a]	29.5 [a]	26.2 [ab]	23.9 [b]
Gel strength (gf)	107 [b]	425 [a]	298 [a]	263 [a]	236 [a]

[a, b]: Means with same superscripts are not different in the same factor (TG treatment or MPI level) (P>0.05).

SDS-PAGE profiles of all treatments with TG addition showed the high molecular weight (biopolymer) and the band intensity of myosin heavy chain might be affected by TG treatment. In addition, the loss of several bands from MPI in TG-mediated MP indicated the interaction of MPI with TG, resulting in it as a substrate for TG. Thus, the formation of biopolymer (arrows in Figure 1) could be explained by the interactions among TG, MPI and MP gel (Figure 1). Chin, Go, & Xiong (2009b) also reported that myosin heavy chain, casein and soy proteins disappeared with TG incubation, contributing to biopolymer formation by gel network.

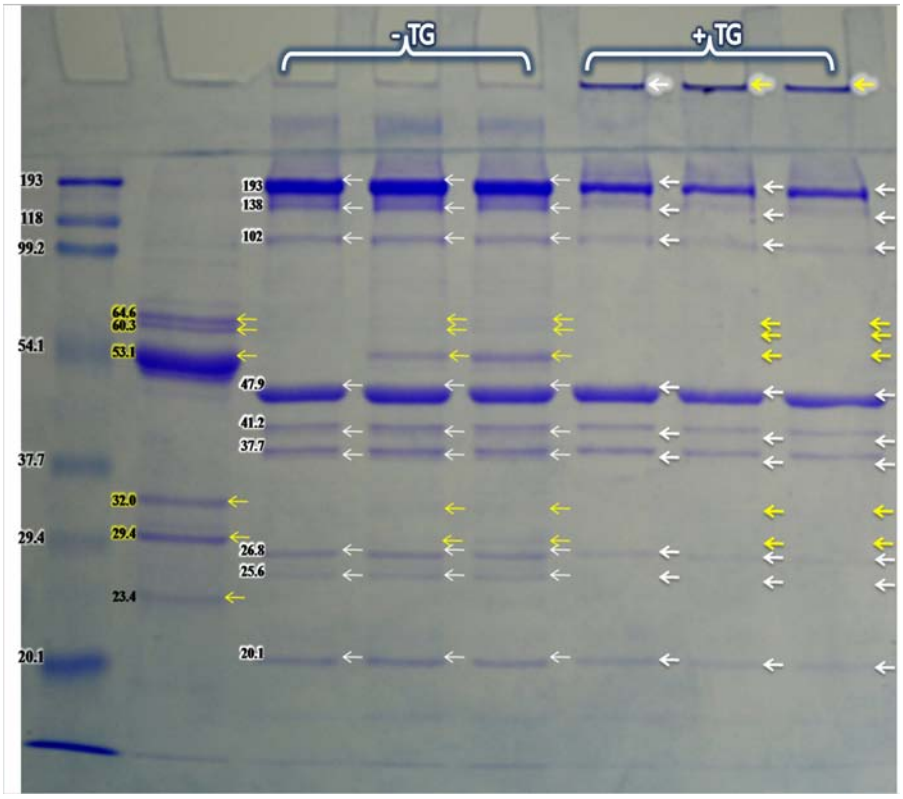

Figure 1. SDS-profile of pork ham myofibrillar proteins (MP) as affected by transglutaminase (TG) treatment and different levels of mungbean protein isolates (MPI): Lane M, standard marker of molecular weight; lane 1, MPI; lane 2, MP; lane 3, MP + 0.27% MPI; lane 4, MP + 0.53% MPI; lane 5, MP + 1% TG; lane 6, MP + 1% TG + 0.27% MPI; lane 7, MP + 1% TG + 0.53% MPI. Arrows indicate visual changes (in bold) in band area or intensity.

The thermograms of MP alone or in combination with MPI (a) or TG and MPI (b) are shown in Figure 2. MP alone (CTL) showed three endothermic peaks at approximately 55, 63, and 74°C, corresponding to myosin heavy chain (MHC; head), myosin light chain (MLC; tail), and actin, respectively (Chin, Go, & Xiong, 2009a; Deng, Rosenvold, Karlsson, Horn, Hedegaard, Steffensen, & Andersen, 2002). The addition of MPI disappeared the endothermic peak of MHC and reduced endothermic peak of MLC (Figure 2, (a)). The thermogram of TG-mediated MP (b) showed reduced peak temperature of MLC (Figure 2, (b)), resulting in the conformational changes (denaturation) of protein for the first time. These results indicated that there was interaction among MP, TG and MPI during gel-setting.

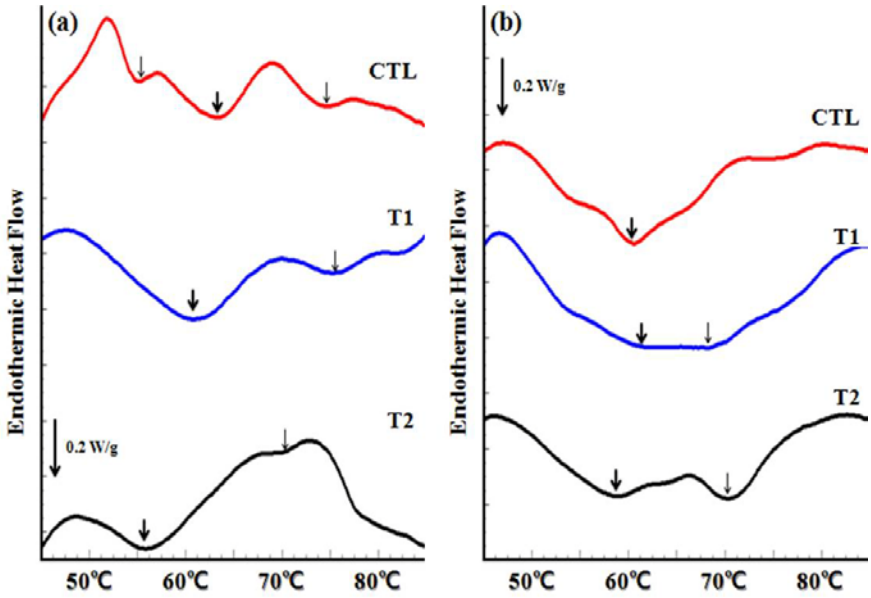

Figure 2. Effects of transglutaminase (TG) treatment and different levels of mungbean protein isolate (MPI) on thermograms of pork ham myofibrillar proteins (MP). Arrows indicated the endothermic peaks of characterized MP (a) without TG and (b) with TG.

Microstructures of heat-induced pork myofibrillar protein (MP) gels are shown in Figure 3. Heat-induced MP gels with TG formed a finely stranded structure which has compacted fibers, but with void spaces (b), as compared to that of control (CTL, a). This structure might be explained that TG-mediated MP had higher gel strength and more cooking loss than CTL (Table 2). On the other hand, the addition of MPI into TG-mediated MP gels showed a conglomerated surface (d), but not strongly affected the surface of MP gels without TG treatment (c). These results indicated that interaction between MP and TG alone or in combination with MPI affected gel structure formation during gelation process.

IV. CONCLUSIONS

TG improved gel strength, but increased the cooking loss of MP gels, while the addition of MPI at higher than 0.53% reduced CL, but did not affect the gel strength of MP gels. Interaction between MPI and TG was shown in SDS-PAGE, based on disappearance of MPI bands (29~32 kDa; 53~64 kDa) in TG-mediated MP. Further study should perform to identify the optimal condition of MPI as a binder to improve gel strength in TG-mediated MP.

ACKNOWLEDGEMENT

This study was financially supported by Chonnam National University in the program, 2010, Gwangju, Korea.

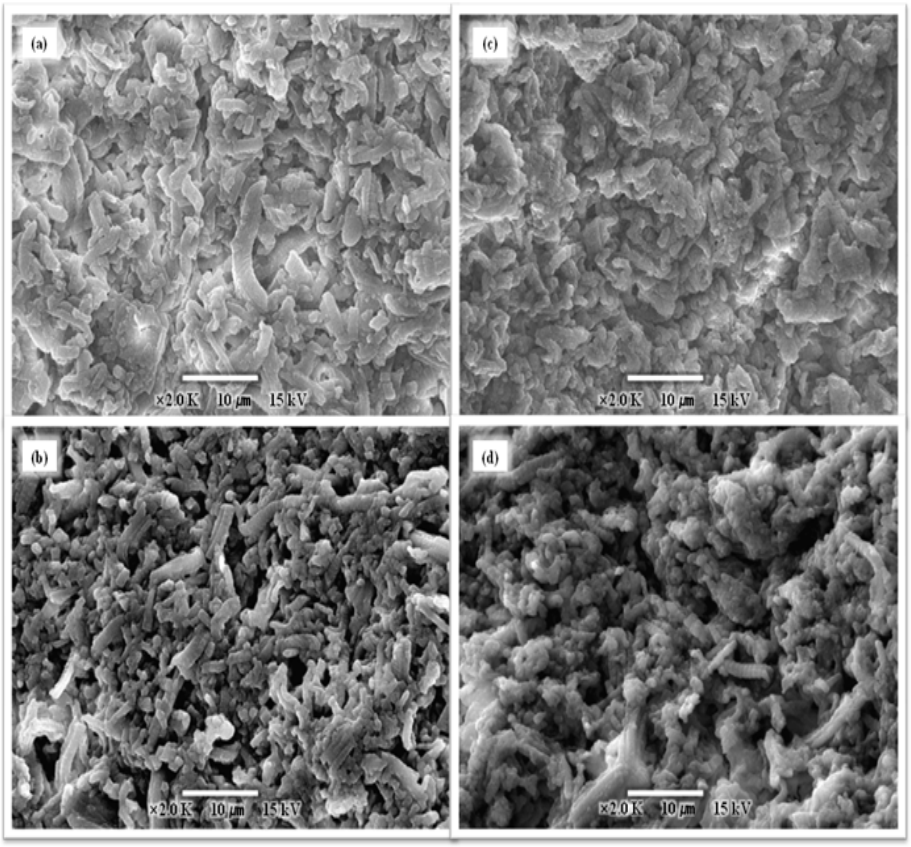

Figure 3. Microstructures of pork ham myofibrillar protein (MP) heat-induced gels (a) with transglutaminase (TG) treatment (b) or mungbean protein isolate (MPI) addition (c), and combinations of MPI as substrates for TG (d) at × 2000 magnification, respectively.

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