EFFECTS OF MUNGBEAN PROTEIN ISOLATES ON MICROBIAL TRANSGLUTAMINASE-MEDIATED PORCINE MYOFIBRILLAR PROTEIN GELS AT VARIOUS SALT CONCENTRATIONS

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Abstract – This study was performed to evaluate the effects of salt concentration on the microbial transglutaminase (MTGase)-mediated, heat-induced protein gels mixed with mungbean protein isolate (MPI) is proved by the changes of protein gel functionality (cooking loss and gel strength), strength of biopolymer formation, and different patterns of microstructural characteristics. The synergistic interactions between MTGase and MPI as a meat and water binder to enhance the protein gel functionality were observed at higher 0.3 M salt concentrations.

Key Words – mungbean protein isolates, microbial transglutaminase, salt concentrations, porcine myofibrillar protein, gel characteristics.

I. INTRODUCTION

Muscle proteins are a good source of amino acid and major component related to flavor, texture, and palatability [1]. Myofibrillar protein (MP) gel functionality is influenced by species, rigor state, fiber type, pH, and salt concentration which define as intrinsic and extrinsic factors [2]. Legume seeds and their proteins can be used in meat processing as a water binding agent [3]. In our previous study, mungbean protein isolate (MPI, 0.53%), which was prepared from non-oil legume seed, was confirmed as a substrate for microbial trans-glutaminase (MTGase) and performed as a water and/or meat binder in the low-fat/salt pork model sausages [4] and porcine MP gels [5]. Thus, this study was performed to investigate the effects of MPI as a meat and water binder on MTGase-mediated porcine MP at various salt concentrations (0.15 ~ 0.45 M).

II. MATERIALS AND METHODS

Porcine myofibrillar proteins (MP) were extracted using modified procedures [6] with several washing steps (0.1 M NaCl and 50 mM NaH_2PO_4 buffer solutions; pH 6.25) as previously reported [7], which were prepared from porcine skeletal muscle cuts were trimmed (*M. semimembranosus* and *M. biceps femoris*). Mungbean protein isolates (MPI) was prepared [5] by isoelectric precipitation method [8], and microbial transglutaminase (MTGase) was donated by Ajinomoto Food Ingredients. Approximately 6 g of MP mixtures were loaded into vials, which were centrifuged at 1000 × g for 15 min and adjusted to the concentration of 40 mg/mL (4.0%, w/v) via biuret method [9]. These samples were stored in a refrigerator for 2 h to react with MTGase [10]. MP gel samples were heated at 75 ^{o}C for 30 min in a digital water bath. They were quickly chilled in an ice bath, and stored in a refrigerator until analyzed. To evaluate the MP gel characteristics, pH, cooking loss (%), and gel strength (gf) were measured by solid-type pH meter, weight of water loss (g), and a compression test with 50 mm/min cross speed, respectively. To identify the interactions between MPI and MTGase-mediated MP during gel setting processes, thermal analysis, gel electrophoresis, and microstructure were investigated. The experiment was performed in triplicates and data were analyzed by two-way ANOVA using PASW statistic 18 program with significance ($P<0.05$). Interaction between factors and main effects were analyzed. Post-hoc analysis was performed using Duncan's multiple range tests to compare among the means.

III. RESULTS AND DISCUSSION

Since no interactions between salt concentrations and ingredients treatment were observed on the pH

and cooking loss (CL, %) of myofibrillar protein (MP) gels ($P>0.05$), mean values for pH and CL (%) were pooled and analyzed individually by factor (Table 1). On the other hand, the interaction between salt concentrations and ingredients treatment was significant on the gel strength (GS, gf) ($P<0.05$) and data by two different factors are shown in Table 2. Reducing salt concentrations led to increased CL (%), and decreased pH values and GS (gf) ($P<0.05$). Microbial transglutaminase (MTGase) treatment increased pH values, CL (%), and GS (gf) of MP gels, while MTGase and mungbean protein isolates (MPI) combination had synergistic effects on the GS (gf) of MP gels ($\geq$0.3 M salt concentration) ($P<0.05$). These results suggested that salt concentrations could aid in forming a stable structure in mixed MP gels. However, MTGase addition reduced the water retention ability of MP after cooking with the production of tight covalent bonds, resulting in the formation of isopeptides and hence excessive water could be extracted out during inter- and intramolecular interactions in cooking process [11]. Based on these results, MPI improved the water retention ability of MTGase-mediated MP gels during the heating process probably by alleviating the electrostatic interactions catalyzed by the MTGase reaction. As results of GS (gf), MTGase would be a good binding agent to enhance the GS (gf) of MP, regardless of salt concentrations. MPI also served as a meat binder and a substrate for MTGase in MP, but its combination effects were limited at a low salt concentration of 0.15 M. Therefore, MP with higher 0.3 M salt was required to enhance the GS (gf) of MP, when it was treated with MTGase alone or combined with MPI. However, those effects will be changed by different conditions of MP gel preparation (e. g. heating rate, MTGase level, and muscle cuts) [5, 12]. Because muscle types responded differently to pH in gel properties and affected storage modulus of salt-soluble proteins [13]. On the other hand, in thermal analysis, the effects of salt concentrations on gel setting were less than that of binding agents (MTGase and/or MPI) in MP (Fig. 1). However, thermograms of 0.3 and 0.45 M had higher enthalpy than 0.15 M for the myosin denaturation of gel setting during the heating process. These results partially due to the solubility of protein which influenced the heat-induced gelation [14]. In SDS-PAGE profiles, the salt concentration did not affect the band intensity of MP; however, increased salt concentrations (>0.15 M) had a noticeable loss of myosin heavy chains (MHC) and myosin light chain (MLC). The intensity of biopolymer reduced with increasing salt concentrations (0.15 M $\rightarrow$ 0.3 and 0.45 M), as shown in the boundary between the stacking gel (Fig. 2). These results indicated that salt concentrations might affect the gel properties of MTGase-mediated MP gel with MPI. Because different solubility of muscle protein (subunits) in the polymerization of MP may affect substrates for MTGase which converted MHC and actin into lower molecular-weight polypeptides that gradually diminished as the ionic strength increased [15]. The major band of MPI was used as a substrate for MTGase activation in polymerization of MP, regardless of salt concentrations. This result suggested that MPI could be used as a substrate for MTGase in MP, even at low-salt concentration (0.15 ~ 0.3 M). Because the cross-linking with MP and the presence of additional protein can accelerate to the enzyme-catalyzed reaction relating to intra-molecular association (isopeptides bond) [15]. In microstructure, the increased salt concentrations affected the three-dimensional structures of MP gels, with changes from strings of beaded strands to clusters and conglomerates tightly glued together, regardless of treatments, while the combination of MTGase and MPI affected the formation of compacted, but void structure and the surface of MP gels was similar patterns, regardless of salt concentrations (Fig. 3). Therefore, the swollen and conglomerated structures of MTGase-mediated MP gels with increased salt and addition of MPI might lead to high gel strength and yield of MP after cooking.

IV. CONCLUSION

Mungbean protein isolate contributed to the meat and water binders on the MTGase-mediated heat-induced porcine myofibrillar protein (MP) gels at salt concentration higher 0.3 M.

Reducing salt concentrations led to decreases in gel strength and cooking yields. Low-salt MP gels showed fragmented structures in microstructures.

Thus, the combination of MTGase and MPI with increased salt concentrations was necessary to improve the meat protein functionality.

Table 1 Pooled means of gel functionality

Parameters	Salt concentration (M)			Treatment		
	0.45	0.3	0.15	CTL	T1	T2
pH	6.43^{a}	6.40^{ab}	6.39^{b}	6.36^{b}	6.42^{a}	6.45^{a}
CL (%)	20.9^{c}	31.0^{b}	41.9^{a}	25.9^{c}	35.6^{a}	31.5^{b}

Factors: MP affected by different salt concentrations and treatment (CTL, MP without MTGase or MPI; T1, MP with 0.6% MTGase alone; and T2, MP with 0.6% MTGase and 0.53% MPI); $^{a-c}$ Means with same superscripts in a same row are not different (P>0.05).

Table 2 Gel strength (gf)

Treatment		Salt concentration (M)		
		0.45	0.3	0.15
CTL	Mean	76.3^{aC}	41.3^{bC}	42.0^{bB}
	SD	15.5	17.2	1.41
T1	Mean	210^{aB}	95.3^{bB}	66.5^{bA}
	SD	7.78	15.3	6.36
T2	Mean	385^{aA}	149^{bA}	65.0^{cA}
	SD	11.3	8.66	7.07

Treatments: as in Table 1; $^{A-C}$ Means with same superscripts are not different in column (P>0.05); $^{a-c}$ Means with same superscripts are not different in row (P>0.05).

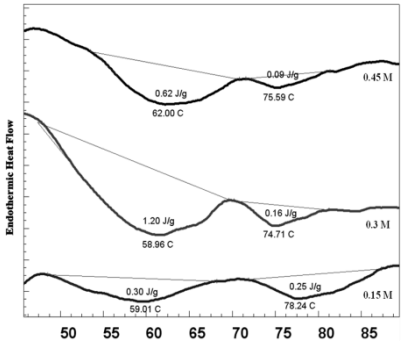

Figure 1. Thermograms

Treatments: MP with 0.6% MTGase and 0.53% MPI at various salt concentrations (0.15, 0.3, and 0.45 M)

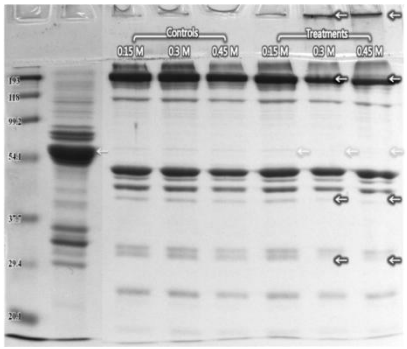

Figure 2. SDS-PAGE profiles.

Lane 1, standard marker; lane 2, MPI; lane 3-5, MP control without MTGase and MPI (CTL) in various salt concentrations (0.15, 0.3, and 0.45 M); lane 6-8, MP treatment with MTGase and MPI (TRT) in various salt concentrations (0.15, 0.3, and 0.45 M), respectively.

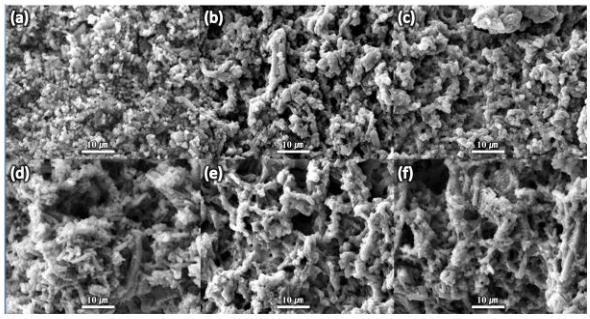

Figure 3. Microstructures.

Treatments: (a) 0.15 M salt MP gels (CTL), (b) 0.3 M salt CTL, (c) 0.45 M salt CTL, (d) 0.15 M salt MP gels with 0.6% MTGase and 0.53% MPI (TRT), (e) 0.3 M salt TRT, (f) 0.45 M salt TRT, at × 2000 magnification, respectively.

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