PE4.73 Effects of Salt and Phosphate Combinations on Rheological Properties of Transglutaminase-mediated, heat-induced Myofibrillar Protein gel 258.00

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Abstract - The objective of this study was to determine the myofibrillar protein mixed gel containing konjac flour and TG combination, and phosphates under different salt levels. Both TG and KF system improved the gel strength and cooking loss, of myofibrillar protein mixed gel, respectively, and the heat gelation was apparent when the salt level was increased from 0.3 to 0.6 M. The partial replacement of ortho with pyrophosphate at the ratio of 3:1 improved the gel strength at 0.6M salt ($p<0.05$), whereas no differences were observed at reduced salt level (0.3 M). The phosphates did not affect the protein profile of MP mixed gel and thermal transition, however, it may decrease voids among the gel matrix, resulting in the production of strong heat-induced gel, as compared to those with orthophosphate alone.

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I. INTRODUCTION

There are many factors affecting to the protein functionality in meat and meat products. Among the factors, salt and phosphate have been widely used in the manufacture of processed meats, such as sausage and hams. Salt improves the water holding capacity and fat binding properties of meat products, as well as giving them desirable texture, improving their sensory properties, and increasing the cooking yield [1] Phosphates are incorporated into the sausage manufacture to increase water binding, resulting in the better textural properties of meat products [2]. In addition, it increased pH to prevent discoloration of meat products [3]. There were classified into different groups, orthophosphate, pyrophosphate, tripolyphosphate, and polyphosphate, based on the number of phosphorous atom. Transglutaminase (TGase, glutamyl-peptide: amine £^-glutamyltransferase, EC 2.3.2.13) is an enzyme to improve the functional properties of meat and meat products. It catalyze acyltransfer reactions between £^-carboxyamide of peptides or protein-bound glutamine residues (acyl donaors) and primary amine (acyl acceptors) and has an ability to cross-link protein through covalent bond between glutamine and lysine (Folk, 1980). Therefore, it has been applied various meat and meat products to function as a binding agent so that the meat may have a viscoelastic texture. Since the TGase activity was affected by several factors, optimum condition of TGase to act with substrates and better condition. Especially, phosphates affect the ultrastructure of myofibrils, resulting in different extractability of muscle protein depending on the type of phosphate [4]. Thus, replacement of ortho phosphate with pyrophosphate at the ratio of 3:1 on the protein functionality of myofibrillar protein mixed gel containing sodium caseinate (SC) and konjac flour and determine the best combination of gel functionality with various salt and phosphate combinations mediated by TGase.

II. MATERIALS AND METHODS

A. Materials

Post-rigor pork meats (Boston but) were purchased from a meat market (Excel¡¦s Smartchoice Corporation, Whichita, KS). The pork meats were trimmed of visible fat and connective tissues, cut into cubes (2 cm3), double vacuum-packaged and frozen until utilized. Microbial transglutaminase (MTGase) used in this study was a crude enzyme preparation (Activa-T1, 99% maltodextrin, 1% MTGase) provided by Ajinomoto Food Ingredients (Chicago, IL, USA). All other chemicals used for this study were at least reagent grade.

B. Sample preparation

The frozen meat was thawed at 4oC for overnight and myofibrillar protein (MP) was extracted using a modified method of Xiong [5]. The pork meat was

washed three times with 4 vol (v/w) of 0.1 M NaCl, 50 mM NaH2PO4 buffer (pH 6.25) and followed by washing with 8 vol (v/w) of 0.1 M NaCl (pH 6.25). Each of the washing step was performed by centrifugation at 2000 x g for 15 min at 2oC. After drained out the supernatant, the pellet protein was adjusted the protein concentration of 4% using Biuret method and bovine serum albumin used as a standard protein [6]. Partially hydrolyzed sodium caseinate (SC, HMP 26, American Casein Company, Burlington, NJ) was prepared with the dissolving with different salt solutions (0.3 vs 0.6 M) at the concentration of 0.53% in the MP suspension. After the MP gel mixture containing SC, 0.27% KF (Nutricol, ME 8721, FMC biopolymer, Pensylvania, PE) and TGase (1%) was mixed with different salt and phosphate combinations (ortho : pyrpphosphate = 3 : 1), gel functionality was investigated as affected by different proportion of phosphates at different salt levels.

C. Gel strength and cooking loss

After mixing with MP gels with SC, KF and TGase, each 5 ml of the mixed protein suspension put into the small vial (Kimble Glass Inc., 1.5 x 5 cm) and heated in isothermeral water bath (model 3013 S, Fisher Scientific) from 20 to 72oC with 1oC/min increments. After heated, the gel was put into ice for a while to cool down and stored at the refrigerator until analyzed. Before analysis, the heat induced gel samples were equilibrated at room temperature for about 2 hr, cooking loss (CL, %) was measured by weighing the differences before and after heating. Gel strength (gf) was performed by the compression the sample axially between two parallel plates in the instron machine (Model 4301, Instron Corp., Canton, MA, USA) to approximately 20% of the original height (80% deformation) at a cross speed 50 mm/min as described by Bourn (1978). A 1 N load cell was used for the measurement and the first peak values presented breaking strength (g) of the gel samples.

D. Rheological analysis

MP mixed gels containing SC, KF and TGase at different proportion of ortho and pyrophosphate combinations under different salt levels were measured to dynamic rheological testing using a rheometer (Bohlin Instruments, Inc., Cransbury, NJ, USA) equipped with two parallel plates of 1 mm apart [5]. Mixed MP gels were produced by heating from 20 to 72oC at a heating rate of 1oC/min. The gelling samples were continuously sheared in an oscillatory mode at a fixed frequency of 0.1 Hz with a maximum strain of 0.02. Changes in the storage modulus (G', i.e. rigidity due to elastic response of the material) were measured with the gelling process with increased temperatures.

E. Sodium-dodecyl sulfate-polyacryl amide-gel electrophoresis (SDS-PAGE)

Protein changes of MP mixed gels as affected by salt level amd phosphate combinations were determined by sodium- dodcyl sulfate-polyacrylamide-gel electrophoresis(SDS-PAGE) according to the Laemmli [6]. A 10% acrylamide separation gel and a 4% acrylamide stacking gel were used. The mixed protein samples were diluted with an equal volume of sample buffer (4% SDS, 2% glycerol, 10% £g-mercaptoethanol, 0.125 M Tris, pH 6.8) and then aliquots of 30 £gL were loaded per well. Electrophoresis was run with a Mini-PROTEAN 3 Cell apparatus (Bio-Rad Laboratories, Hercules, CA, USA).

F. Scanning electron microscopy (SEM)

Three dimensional structures of myofubrillar protein mixed gel were performed according to the modified method of Chin et al. [7]. Samples were prepared at the size of 3 x 3 x 3 mm2 and fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (PBS) at 4oC for 24 hrs. The fixed samples were washed with three times of 0.1 M PBS for 10 min and then post-fixed with 1% Osmium tetraoxide in PBS for 5 hrs at refrigerated temperature. After washed with PBS three times, the samples were dehydrated with incremental concentration of ethanol from 50 to 100%. Dried samples were coated with gold palladium and examine the three dimensional structure with SEM machine (Hitachi High-Technologies Corp., Tyoko, Japan) with 15 kV accelerated voltage. The magnification was varied from 250 to 1000 for the maximum resolution

G. Statistical analysis

The experiment was replicated three times, and the data were analyzed using two-way analysis of variance (ANOVA) in SPSS 12.0 software for Windows with factors for salt concentration (0.3, and 0.6 M) and the replacement of ortho with pyrophosphate. If the interaction between salt concentration and phosphate combination was significant, data were separated out by pyrophosphate treatments within each salt concentration. If the interaction was not significant ($P > 0.05$), data were pool to test the main effect using Duncan's multiple range tests.

III. RESULTS AND DISCUSSION

Since the interaction between phosphate effect, and KF and TG combinations were not significant ($p>0.05$), the data were pooled by phosphate effect and treatments at each salt level.

A. Gel strength and cooking loss

At a 0.3 M salt level, the phosphate effect was not effective, however, the partial replacement of orthophosphate (P) with pyrophosphate (PP) increased gel strength at salt level of 0.6M (Table 1). These results indicated that the phosphate effect of gel strength was interacted with KF and TG combinations. The addition of KF decreased cooking loss at 0.3 M salt, whereas no differences in cooking loss were observed at 0.6 M salt, due to high salt level. These results agreed with previous report that the KF improved the water binding capacity of TG-mediated MP gels [8]. However, the TG-mediated MP mixed gel increased the gel strength, as compared to those without TG. These results were also confirmed the previous report that the increased salt level increased the gel strength and the TG effect was improved with increased salt levels [8]. Xiong et al. [4] reported that the ultrastructure of myofibrils and extraction of their constituents in the order pyrophosphate (PP) *f*-tripolyphosphate (TPP) > hexametaphosphate (HMP) > orthophosphate (P)= nonphosphate control. They also reported that the addition of Mg2+ might play a critical factor to dissociate of actomyosin by PP which had little effect on myofibril swelling and myosin extraction.

B. Rheological properties

If TG was not added to the MP mixed gel, the storage modulus (G) of MP gels started to increase after 50oC and reached approximately a maximum value of ~1000 Pa (Figure 1). However, the G,,S increased rapidly and reached up to 2500 to 3000 psi for 0.3 M salt and 5000 to 5500 pa for 0.6 M salt, when TGase was added (Figure 1). These results indicated that TG was activated MP mixed gel, resulting in increases of G,,S. Increased salt level increased G,,S values, whereas the partial replacement of orthophosphate with pyrophosphate didn't affect G,,S and the effect of KF on the G,,S decreased to those without TGase. Robe and Xiong [9] reported that the addition of PP and tripolyphosphate (TPP) reduced G,,S to a similar extent for all three muscle types and the effect of phosphate on protein gelation was not limited to increasing ionic strength and altering pH. They also reported that the effect of TPP on protein gelation was not solely to ionic strength and pH at the ionic strength level examined. In this study, at 0.3 M salt, slight increased G,,S values were observed, but decreased G,,S values were observed with replacement of PP at 0.6 M salt. Thus, the replacement of orthophosphate with pyrophosphate interacted with the salt concentrations. .

C. Sodium-dodecyl sulfate-polyacryl amide-gel electrophoresis (SDS-PAGE)

During incubation at 4oC, SDS-PAGE was performed to determine the changes of protein bands as affected by PP and treatment effect under different salt levels. No differences in SDS-PAGE gel pattern were found with the addition of konjac flour and partial replacement of ortho-phosphate with pyrophosphate (Figure 2). The increased salt level and incubation time made protein bands containing 32-34 kDa disappeared rapidly [7]. These protein bands might be probably from the sodium caseinate, resulting in the involving in the cross liking induced by TGase.

D. Scanning electron microscopy (SEM)

The scanning electron microscopy of gel structure as affected by phosphate and KF and TG treatments were investigated under different salt levels (Figures not shown). The MP gel alone had several cavities in the gel network and the added konjac flour inserted into the protein matrix, resulting in a little bit soft texture [7]. However, the addition of TGase made the gel structure more compact [8]. This result may be partially due to the covalent crosslinking induced by TGase. The increased salt content increased the gel matrix swollen, resulting in more compact gel structure. The partial replacement of orthophosphate with orthophosphate also decreased voids among the gel matrix, resulting in the production of strong heat-induced gel, as compared to those with orthophosphate alone. Xiong et al.[4] reported that the increased protein solubility was occurred at the 0.3 and 0.4 M salt resulted from release of myosin, however phosphate effect diminished at the salt level higher than 0.6 M salt.

IV. CONCLUSION

Both TG and KF system improved the gel strength and cooking loss of myofibrillar protein mixed gel, respectively, and the effect was apparent when the salt level was increased from 0.3 to 0.6 M. The partial replacement of ortho with pyrophosphate at the ratio of 3:1 improved the gel strength at 0.6 M salt, whereas no differences in gel strength were observed at reduced

salt level (0.3 M). The phosphates did not affect the protein profile of MP mixed gel and thermal transition, however, it may decrease voids among the gel matrix, resulting in the production of strong heat-induced gel, as compared to those with orthophosphate alone.

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