PE4.45 Effects of sodium alginate and transglutaminase as a cold-set gelling system on myofibrillar protein gelation characteristics 155.00

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***Abstract*— this study was investigated individual and combined effects of microbial transglutaminae (TG) and sodium alginate (SA) systems on cold-set myofibrillar protein (MP) gelation characteristics at various salt levels. Apparent viscosity and gel strength data revealed that low salt concentration (0.1 M) favored the activation of SA systems, while TG system contributed the MP gel characteristics at high salt levels (0.3 M). Meanwhile, TG combined with SA system was not affected by salt concentrations. In consequence, SA system contributed to improve water binding property and cold-set MP gel formation, while TG system enhanced the textural properties of MP gel after cooking at low salt levels. In addition, TG effects on textural properties of MP gel were dominant at high salt levels, and SA system complemented TG induced moisture loss. Therefore, the results implicated that TG combined with SA system had a potential advantage in improving the quality of cold-set meat products.**

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I. Introduction

There are already numerous studies regarding on application of either TG or SA as a cold-set meat binder or gelling system in model or actual meat products. Meanwhile, most of them investigated TG or SA individually, leading to a difficulty to directly compare the effects of TG and SA. Sukulim et al. [1] investigated the TG and SA effects on physical properties of restructured scallops without salt addition, and reported that SA showed the highest binding strength after 2 h setting time, as compared to TG after 24 h. In addition, maximal binding strength was higher at SA treatment than TG. On the other hand, Moreno et al. [2] compared the restructured fish added TG and SA at 1.5% NaCl concentration, and obtained 1.20 and 1.91 N of maximal breaking force at SA and TG treatment, respectively. The results were obviously showed the salt effects in SA and TG systems. Therefore, this study was aimed to compare the individual and combination effects of TG and SA systems on cold-set gelation characteristics of porcine MP at various salt levels, and to establish an independent MP gelling system from various potential factors.

II. Materials and Methods

A. Materials

Porcine *M. longissimus dorsi* was trimmed all visible fat and connective tissue, and cut into 1 cm cubes. The meat cubes were divided into 200 g portions, vacuum-packed with polyethylene pouch, and frozen at -70°C prior to use. Microbial TG (Activa-TI, 1% TG with 99% maltodextrin) was provided by Ajinomoto Food Ingredients (Chicago, IL, USA), and partially hydrolyzed sodium caseinate (SC, HMP 26) was provided by American Casein Company (Burlington, NJ, USA). All other chemicals were analytical grade.

B. Sample preparation

Frozen meat was thawed at 4°C for overnight, and MP was extracted by the method of Chin et al. [3]. MP sol was prepared by adjusting the protein concentration to 4% (pH 6.25). TG system consisted with 0.6% TG and 0.5% SC, and SA systems with 0.5% SA, 0.2% calcium carbonate (CC) and 0.5% glucono-δ-lactone (GdL). For analysis, 5 g aliquots of samples were loaded into glass tubes (Ø 12 mm), and incubated for up to 24 h at 4°C.

C. Rheological analysis

Rheological changes during gel-setting were measured using a concentric cylinder type rotational rheometer (RC30, Rheotec Messtechnik GmbH, Berlin, Germany). The sample was sheared by linearly increasing shear rate from 0 to 300/s for 60 s. Obtained shear rate-stress data were fitted by power law model.

D. Gel strength and yield

Half of cold-set MP gels incubated at 4°C for 24 h were compressed to failure of gel using an Instron universal testing machine (3340, Instron Corporation, Canton, MA, USA) with a stainless steel probe (9 mm

diameter). The rest of gels were heated by increasing temperature from 0 to 75°C at 3°C/min increment using a thermostat. After heating, the exudates in gels were discarded. Cooking yields was determined by assessing the weigh of gels, and gel strength was measured by the same method of cold-set ones.

E. Gel electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to identify the MP polymerization after 24 h of incubation. A 9% acrylamide separating gel and a 4% acryl amide stacking gel were used with a Mini-PROTEAN 3 Cell apparatus (Bio-Rad Laboratories, Hercules, CA, USA).

F. Statistical analysis

The data were analyzed by two-way ANOVA using the SAS Statistical Program 9.1 (SAS Institute, Cary, NC, USA). Main factors included salt concentrations (0.1, 0.2 and 0.3 M) and combinations of MP with SA and TG. Differences among the means were compared using Duncan's multiple range test.

III. RESULTS AND DISCUSSION

A. Apparent viscosity

All treatments showed an exponential increment in apparent viscosity during incubation (Fig. 1). SA system showed the best gelation kinetics at 0.1 M salt concentration as compared to those containing higher salt level. However, increasing salt concentration decreased gelation kinetics of SA system. In addition, SA system could not form a gel within 24 h of incubation at 0.3 M salt concentration, indicating that SA system was favorable at relatively low salt level (< 0.2 M). In contrast, TG system had no effect on MP gelation during 24 h incubation at up to 0.2 M salt concentrations. However, rapid MP gelation was shown with increasing salt concentration (> 0.3 M). The result revealed that TG system required at least 0.3 M of salt to form a cold-set MP gel. The TG mediated cold-set MP gelation was mainly due to covalent bonds between lysyl and glutamyl residues and thereby the bonding intensity was dependent on MP solubilization [4]. The TG combined with SA system showed a similar MP gelation kinetics and was not affected by salt concentrations, while increasing salt level tended to shorten the gel forming time. The results indicated that SA system was predominant at low salt level (< 0.1 M) and lost rapidly their activity with increasing salt concentration, while increasing salt level also improved the activity of TG system resulting in acceptable MP gel formation regardless of salt levels.

B. Gel strength

For cold-set MP gels (Fig. 2a), MP alone could not form a gel regardless of salt concentration. For TG system, increasing salt level increased the cold-set MP gel strength ($p<0.05$), and the palatable gel strength was obtained at 0.3 M salt level. In addition, gel strength of MP gel containing TG system was further increased after thermal treatment (Fig. 2b), indicating that TG system was activated during thermal processing. Generally, it was accepted that optimal activation temperature of TG was 37°C [5], while their activity varied with substrate sources and pH. Optimal

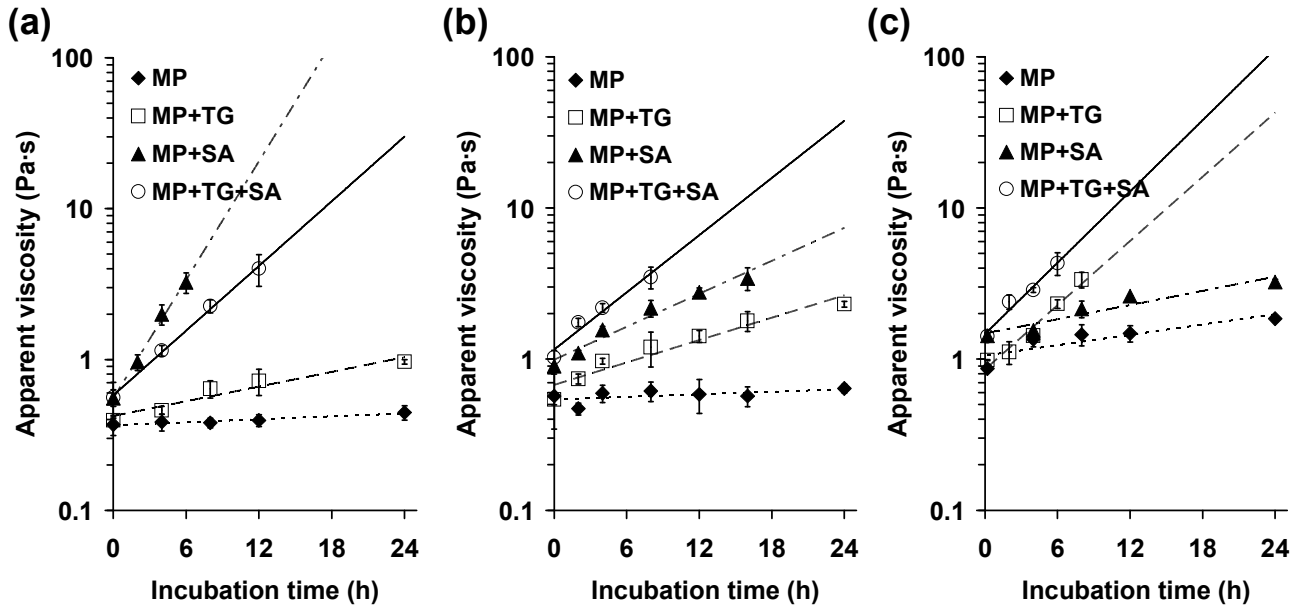

Fig. 1. Changes in apparent viscosity of myofibrillar protein (MP) containing (a) 0.1 M, (b) 0.2 M and (c) 0.3 M salt during 24 h of incubation. TG and SA represented transglutaminase and sodium alginate systems, respectively.

reaction condition of TG with SC was reported at 50°C and pH 9 [6]. Although, the TG added cold-set MP gel strengths at less than 0.2 M salt were not enough for palatable gel strength (50 g_f), heat treatment improved the texture of MP gel even if low salt was added (< 0.2 M). From the above results, TG system could be added into either highly salted meat products (> 0.3 M) to form a cold-set MP gelation, or cooked meat products containing low salt (< 0.2 M) to improve the textural properties after cooking.

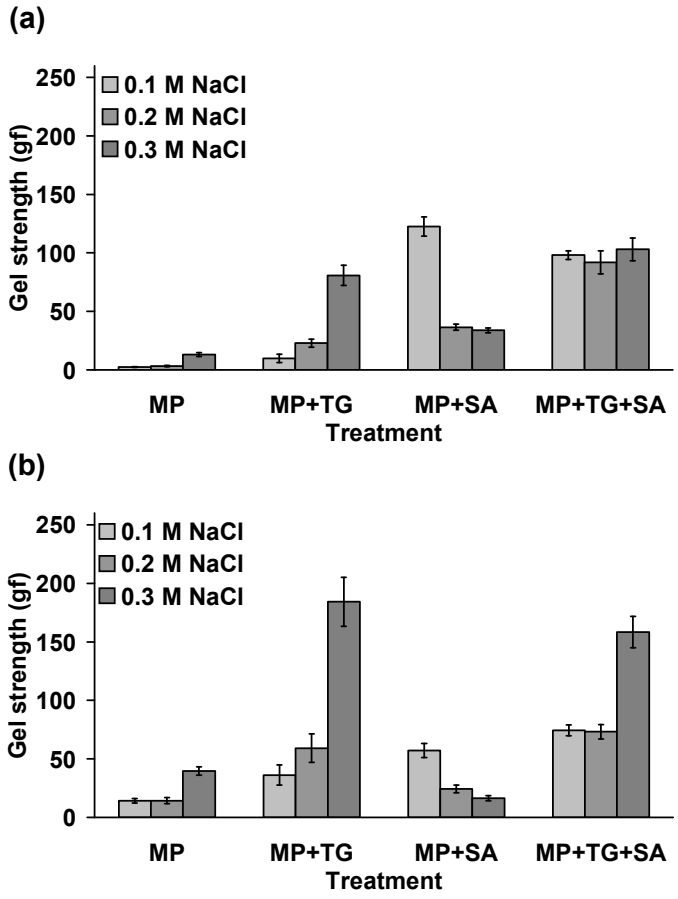

Fig. 2. Effects of transglutaminase (TG) and sodium alginate (SA) systems on gel strength of (a) cold-set and (b) heat treated myofibrillar protein (MP) gel.

Meanwhile, SA system showed the highest gel strength among treatments at 0.1 M salt level, however, decrement in gel strength was found above 0.2 M salt ($p<0.05$). Furthermore, SA system mediated cold-set MP gel was lost their gel strength after thermal treatment. The textural degradation of MP gel after cooking could be therefore explained that SA which was not converted into gel interpreted interactions between MPs during thermal treatment, and provably led to low gel strength after cooking. Therefore, the usage of SA system to form a cold-set gel could be limited to meat products containing low salt (0.1 M).

In consequence, the TG and SA system were highly associated with salt levels. The combination treatment of TG and SA showed no different with increased salt levels. On the other hand, gel strengths of combination treatments of TG and SA were changed after thermal treatment. At less than 0.2 M salt concentration, the gel strengths of combination treatments were lower than cold-set ones, although no difference between salt levels was shown ($p>0.05$). However, combination treatment containing 0.3 M salt showed higher gel strength than below 0.2 M salt concentrations after cooking ($p<0.05$). Based on the results, the thermal treatment affected the gel strengths of combination treatments, i.e., their gel strengths were reduced up to 0.2 M salt level ($p<0.05$), however, increased gel strength was observed at 0.3 M salt level, indicating the major contributors between TG and SA systems.

C. Yield

All treatments showed an increase in cooking yield with increasing salt level ($p<0.05$), with the exception of combination treatments between 0.2 and 0.3 M salt (Fig. 3). Among the same salt level, TG system showed the lowest cooking yields ($p<0.05$), indicating the detrimental effects on protein-water interactions. In general, TG mediated covalent bonding in MPs was the most strong interaction as well as ion-ion interactions [7]. The MPs had, therefore, more affinity with the protein moiety than surrounded water molecules when TG formed crosslink among proteins, led to low cooking yield. Therefore, the addition of hydrocolloids with TG system would be beneficial in yield aspects [3]. SA system improved the cooking yield when comparing other treatments ($p<0.05$) even at low salt (0.1 M). It was generally identified that SA had high water-binding properties due to highly hydrophilic nature of SA. On contrast, the water-binding property of SA was reduced when calcium ion was attached in binding sites of SA by syneresis [8]. However, the phenomenon was recovered either by addition of higher CC or by concurrent presence of a monovalent cation [9]. Moreover, GdL itself reduced the pH of MP sol due to slow dialysis of acid. However, Draget et al. [10] suggested that balancing CC and GdL at the molar ratio 1:2 could make a uniform SA gel at neutral pH without syneresis. In the current study, GdL was interacted with equivalent CC, and thus did not reduce the cooking yield of MP.

Meanwhile, combination treatments showed lower cooking yields than those of SA, but higher than TG alone treatment ($p<0.05$), indicating potential advantage of combined application of TG and SA system. Only cooking yield of SA and TG combination treatment at 0.3 M salt level was lower than control ($p<0.05$), probably due to the enhanced protein-protein

interactions through TG activation.

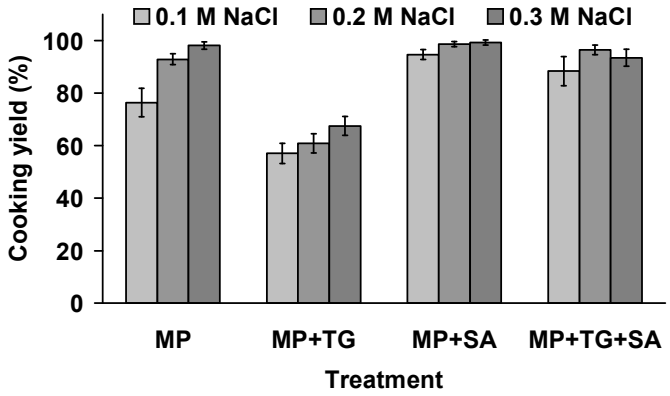

Fig. 3. Effects of transglutaminase (TG) and sodium alginate (SA) systems on cooking yield of heat treated myofibrillar protein (MP) gel.

D. Gel electrophoresis

For SDS-PAGE profiles (Fig. 4), all TG added treatments (TG and combination) showed high molecular weight component in the boundary between stacking gel and separating gel, regardless of salt level. The band intensity of MHC was not affected by TG treatment at 0.1 M salt level, however, the band area of MHC was reduced with increasing salt levels. The result was supported the TG mediated polymerization of MP, particularly MHC, to form a gel. In addition, 42 kDa component corresponding to troponin T was disappeared by TG treatment which also implicated an interaction of troponin T as a substrate of TG [11]. From the result, TG mediated MP cold-set gelation could be estimated the result of interaction among TG, SC and MP. On the other hand, SA system showed no significant changes in SDS-PAGE pattern. This was obvious evidence of no interaction between SA and MP. In most case, anionic polysaccharides can interact with MP if polyvalent cations are presented [7]. However, calcium-induced SA gelation occurred in G-block segment in SA, and thus, formed their own gel network.

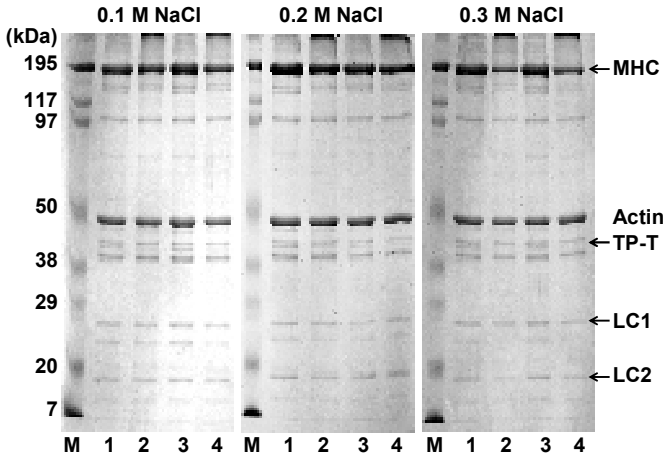

Fig. 4. Effects of treanglutaminase (TG) and sodium alginate (SA) systems on SDS-PAGE profiles of myofibrillar proteins (MP). MHC, LC and TP-T indicated myosin heavy chain, myosin light chain and troponin T, respectively. Lane M, molecular weight marker; lane 1, MP; lane 2, MP+TG; lane 3; MP+SA; lane 4, MP+TG+SA.

IV. Conclusion

In the current study, the effects of two main cold-set gelling systems, TG and SA, on gelation characteristics of MP showed definitely salt concentration-dependent. From the products point of view, individual usage of these cold-set gelling systems had no effects on physical and functional properties of final products, since salt level used in the formulation vary with the products. By emerging the two cold-set gelling systems, cold-set meat products with high moistness and enhanced textural properties after cooking could be produced without salt-dependency.

Acknowledgement

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