Konjac flour improved textural and water retention properties of transglutaminase mediated, heat–induced porcine myofibrillar protein gel containing sodium caseinate

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Abstract

Functional properties of heat-induced gels prepared from microbial transglutaminse (TG)-treated porcine myofibrillar protein (MP) containing sodium caseinate with or without konjac flour (KF) under various salt concentrations (0.1, 0.3 and 0.6 M NaCl) were evaluated. The mixed MP gels with KF exhibited improved cooking yields at all salt concentrations. TG treatment greatly enhanced gel strength and elasticity (storage modulus, G´) at 0.6 M NaCl, but not at lower salt concentrations. The combination of KF and TG improved the gel strength at 0.1 and 0.3 M NaCl and G´ at all salt concentrations, when compared with non-TG controls. Incubation of MP suspensions (sols) with TG promoted the disappearance of myosin heavy chain and the production of polymers. The TG-treated MP mixed gels had a compact structure, compared to those without TG, and the KF incorporation modified the gel matrix and increased its water-holding capacity. Results from differential scanning calorimetry suggested possible interactions of MP with KF, which may explain the changes in the microstructure of the heat-induced gels.

Introduction

Consumers prefer to select the healthier-foods with reduced salt and fat, and improved sensory and textural characteristics. Understanding the interaction of meat protein and other ingredients would be critical to manufacture better products. Myofibrillar protein (MP) plays an essential role in producing the desirable texture and binding water in comminuted meat products, such as sausages. To bind the meat protiens and other ingredients with reduced fat and salt, newer technologies or ingredients should be developed. Transglutaminase (TG) is an enzyme that catalyzes acyltransfer reactions between γ-carboxyamide of peptides or protein-bound glutamine residues (acyl donors) and primary amines (acyl acceptors). The combination of muscle and dairy proteins mediated by TG increased water-holding capacity and produced a firm texture after cooking. In addition, konjac could be also used to improve the textural characteristics and water binding capacity of low-fat meat emulsion products (Chin et al., 1998a). The objective of the present study was to evaluate the effect of KF on the rheological properties of TG-treated pork MP under various salt concentrations.

Materials and methods

Fresh pork shoulders were obtained from a commercial meat packing company. After deboning, the meat was trimmed of visible fat and connective tissues, cut into cubes (~2 cm^3), randomly mixed and then double vacuum-packaged. Microbial transglutaminase (TG), partially hydrolyzed sodium caseinate (SC, HMP 26), and konjac flour (KF, Nutricol ME 8721) were used for this study. Myofibrillar protein (MP) was isolated and purified at 2 ºC according to Xiong (1993). The extracted protein concentration of the final MP pellet was determined by the biuret method (Gornall et al., 1949).

MP pellet was suspended (40 mg/mL protein) in various salt solutions (final NaCl concentrations of 0.1, 0.3 and 0.6 M) containing 50 mM sodium phosphate (pH 6.25). To all the MP suspensions, SC was also added (0.53% w/v). The MP:SC in the gelling solute was 15:2, which mimics a typical emulsified-sausage. For TG-treated gels, the Activa TI powder was added to the gelling solution, at a 1 g/100 g final enzyme concentration, immediately prior to heating. Both TG-treated and control gelling solutions were incubated for 0 or 2 h at 4 C. For gelation, 5-mL aliquots of control and KF- and TG-treated MP gelling solutions were placed into small vials (1.5 cm inner dia × 5 cm height), incubated for 0 and 2 h, and then heated in a programmable water bath from 20 to 72 ºC at 1 C/min increments. After heating, the gels were immediately chilled in an ice slurry and then kept in a 4 ºC refrigerator overnight before analysis. Cooking loss (CL, %) was measured by weighing the samples before (sol) and after (gel) heating, and was expressed as weight difference divided by the original weight (%). Gels in the glass vial were extruded with a stainless steel probe (12.5 mm dia) and gel strength (N) was measured using Instron universal testing machine (Model,

4301, Canton, MA, USA). The rheological properties of MP mixed samples during thermal gelation were measured with a rheometer. SDS-PAGE was performed to identify protein changes in MP gelling sol after incubation with TG for 2 h (Laemmli, 1978). The influence of KF on the thermal stability of MP in the presence or absence of TG was determined using a different scanning calorimeter (DSC) machine. The microstructure of MP mixed gels was examined with a Hitachi-S-800 field emission scanning electron microscope(SEM).

Results and discussion

In the absence of KF, the cooking loss (CL, %) was reduced by increasing salt concentration ($P < 0.05$). When KF was added to the MP gel formulation, it completely prevented moisture loss from cooked gels, even in those prepared at low salt concentrations (0.1 and 0.3 M NaCl). The TG treatments did not affect ($P > 0.05$) the CL of the MP gels formed with 0.1 or 0.6 M NaCl in the absence of KF (Table 1). However, the TG treatment increased ($P < 0.05$) CL of gels formed in 0.3 M NaCl. In 0.1 and 0.3 M NaCl, the addition of TG and KF alone did not affect the gel strength ($P > 0.05$); however, the combination of TG and KF increased the MP gel strength (Table 1). The results indicated that KF likely had a synergistic effect with TG in low salt concentrations. On the other hand, at 0.6 M NaCl, while the presence of TG markedly increased the MP gel strength value, the addition of KF produced no further beneficial effects. Furthermore, incubation time did not appear to have a significant effect on the enzyme action.

Table 1. Cooking loss and MP gel strength as affected by konjac flour, TG and incubation time

		MP (Control)		MP+KF		MP+TG		MP+KF+TG	
Parameters	Salt level	0	2 h	0	2 h	0	2 h	0	2 h
Cooking loss (%)	0.1 M	56.2^{aA}	56.6^{aA}	-	-	56.9^{aA}	54.9^{aA}	-	-
	0.3 M	42.8^{bA}	44.0^{bB}	-	-	51.3^{aA}	47.3^{abA}	-	-
	0.6 M	15.0^{aB}	18.0^{aC}	-	-	11.6^{aB}	20.0^{aB}	-	-
Gel strength (N)	0.1 M	0.27^{cA}	0.29^{cA}	0.41^{cA}	0.43^{bcA}	0.32^{cB}	0.37^{cB}	0.63^{abB}	0.71^{aB}
	0.3 M	0.33^{cdA}	0.28^{dA}	0.45^{bcdA}	0.36^{cdA}	0.51^{bcdB}	0.54^{abcB}	0.67^{abB}	0.77^{aB}
	0.6 M	0.43^{bA}	0.42^{bA}	0.54^{bA}	0.53^{bA}	1.73^{aA}	2.47^{aA}	1.85^{A}	1.80^{aA}

[a-d] Means in the same row having a same superscript are not significantly different ($P < 0.05$).
[A-C] Means in the same column having a same superscript are not significantly different ($P < 0.05$).
-: amount negligible (< 1%).

In the absence of KF and TG, storage modulus (G′) of the MP suspension at 0.1 M NaCl did not change, and the value essentially stayed flat over the heating period (20–72 ºC). When KF was added to the MP suspension prior to heating, the G′ started to significantly increase after being heated to above 55 ºC. However, the incorporation of TG into the MP suspension, irrespective of the presence of KF, greatly promoted gelation, Raising the NaCl concentration from 0.1 to 0.3 M brought about a stronger gelling capability of MP (Fig. 1B). When the salt concentration was further increased to 0.6 M, a much greater gel-strengthening effect was produced by TG, KF and their combinations when compared with 0.1 or 0.3 M NaCl conditions (Fig. 1C). Thus, TG alone was a good gel-promoting agent at high salt concentration (0.6 M), but was less effective at low salt conditions. The results agreed with the report by Trespalacious and Pla (2007).

Salt concentration did not have a pronounced effect on the overall SDS-PAGE pattern of the MP samples except that in samples treated with TG at 0.1 M NaCl, the casein bands disappeared rapidly (lanes 5-8). Increasing the salt concentration delayed the disappearance of the casein (Fig. 2B, 2C). The decreased MHC band intensity corresponded to the appearance of high molecular weight bands that could not enter the resolving gel and thus, stacked on the top of the gel. However,, the 2-h incubation at 4 ºC caused a quite noticeable loss of myosin heavy chain (MHC) in TG-treated MP samples at all salt levels (lanes 6 and 8).

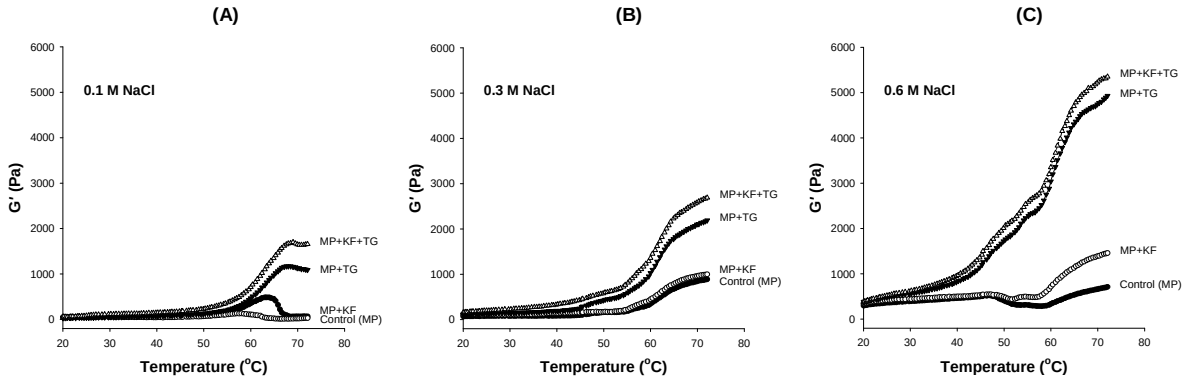

Figure 1. Representative rheograms of heat-induced MP gels (pH 6.25) as affected by konjac flour (KF) and microbial transglutaminase (TG, 2 h incubation) at various salt concentrations. (A) 0.1 M NaCl; (B) 0.3 M NaCl; (C) 0.6 M NaCl.

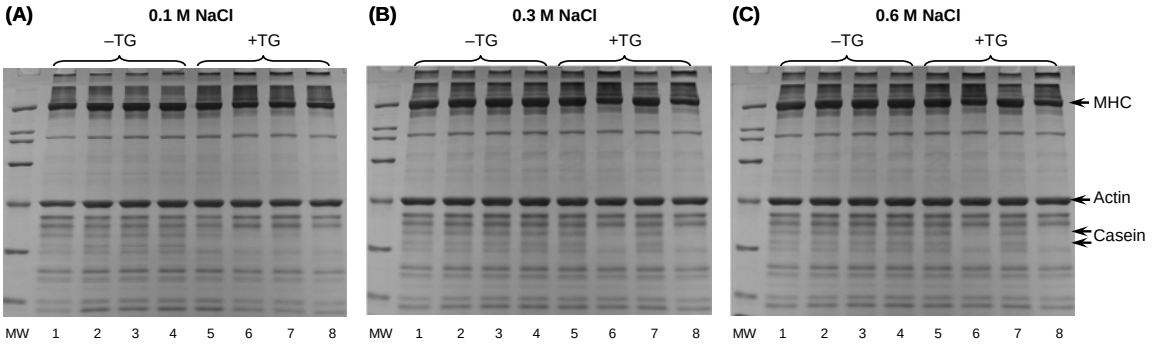

Figure 2. SDS-PAGE profile of MP suspensions (pH 6.25) treated with konjac flour (KF) and microbial translutaminase (TG) at various salt concentrations for 0 and 2 h. (A) 0.1 M NaCl; (B) 0.3 M NaCl; (C) 0.6 M NaCl. Lane MW: molecular weight markers; lanes 1 (0 h), 2 (2 h), 5 (0 h), 6 (2 h): -KF; and lanes 3 (0 h), 4 (2 h), 7 (0 h), 8 (2 h): +KF.

At 0.3 M NaCl, the control MP sample showed three endothermic peaks at approximately 60, 68 and 72 ºC , which corresponded to the denaturation of myosin head, myosin tail, and actin, respectively (data not shown). MP suspensions in 0.6 M NaCl, which formed strong gels upon heating, did not produce characteristic thermal transition (data not shown). This may be because high-concentration NaCl disrupted the ionic bonds in MP (Quinn, et al., 1984). In mocrostrucuture (data not shown), the control MP gel appeared slightly porous and consisted of protein aggregates. Gels with KF not appreciably differ from the control gels and the void spaces remained visible. Cross-linking with TG reduced the empty spaces, but the combination of TG and KF changed the aggregate gel structure into a more or less fibrous gel network. Overall, the microstructural comparison between gels formed at 0.3 and 0.6 M NaCl indicated no remarkable differences.

Conclusions

Through the application of TG and KF and manipulation of incubation time, it is possible to produce well-structured and firm MP gels with minimal cooking loss even at a reduced-salt level.

References

Chin, K. B., Keeton, J. T., Longnecker, M. T., & Lamkey, J. W. (1998a). *Journal of Food Science*, 63, 801-807.

Gornall, A. G., Bardawill, C. J., & David, M. M. (1949). *Journal of Biological Chemistry*, 177, 751-766.

Laemmli, K. E. (1970). *Nature,* 227, 680-685.

Quin J. R., Raymond, D. R., & Harwalker. (1980). *Journal of Food Science*, 45, 1147-1149

Trespalacious, P., & Pla, R. (2007). *Food Chemistry*, 100, 264-272.

Xiong, Y. L. (1993). *Journal of Food Biochemistry,* 16(4), 217-227.