Evaluation of Salt, Microbial Transglutaminase and Calcium Alginate on Protein Solubility and Gel Characteristics of Porcine Myofibrillar Protein

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Abstract

Response surface methodology was adopted to model and optimize the effects of microbial transglutaminase (TG) and calcium alginate (CA) systems of various ratios on the gelation characteristics of porcine myofibrillar protein (MP) at various salt levels. The CA system consisting of sodium alginate (SA), calcium carbonate (CC) and glucono- δ -lactone (GdL) showed no remarkable changes in the salt-soluble fraction, and only minor effects on electrostatic interactions were observed. Increasing CA concentration caused acid-induced hydrophobic interactions in MPs, resulting in increased MP gel strength. The TG system, containing TG and sodium caseinate (SC), induced cold-set MP gelation by formation of covalent bonding. The main advantage of the combined system was a higher cooking yield when the MP gel was heated. These results indicated that 0.7% TG combined with 0.8% CA system can form a viscoelastic MP gel, regardless of salt levels.

Key words: Microbial transglutaminase, calcium alginate, myofibrillar protein, gel characteristics

Introduction

Protein gelation techniques are related to the physical and chemical properties of protein based gel type foods such as cheese, surimi and sausage (Boles and Shand, 1998; Park, 2000). In the last few decades, numerous procedures in protein gelation have been introduced, and some chemical ingredients, including microbial transglutaminase (TG), calcium alginate (CA) and fibrinogen/thrombin (Boles and Shand, 1998), have been available to form a cold-set gel.

In most cases, TG was intensively investigated as a cold-set gelling system. The TG mediated meat binding or protein gelling mechanisms have been published (Folk, 1980; Ramírez-Suárez and Xiong, 2003; Chin *et al.*, 2009a). Nielsen *et al.* (1995) investigated the effects of F XIIIa, a TG from plasma, on cold-set meat binding and reported that the enzyme had the highest activity at 37°C, while no or minor effects at refrigerated temperature. However, this endogenous TG is Ca²⁺-dependent for their activity, and therefore, microbial TG is easier to use in

food processing (Park, 2000). Kuraish *et al.* (1997) postulated that TG system, which consisted of microbial TG and sodium caseinate (SC) as a substrate, could restructure the meat particles at refrigeration temperature without salt or cooking, whereas, most of research reported that TG activity on meat binding was salt-dependent (Ramírez *et al.*, 2002; Küttemeyer *et al.*, 2005; Chin *et al.*, 2009a). These inconsistent results were probably dependent on processing conditions, the source of TG, type of substrates or sample materials as well as activity of TG itself (Nielsen *et al.*, 1995; Kuraish *et al.*, 1997; Ramírez *et al.*, 2002; Chin *et al.*, 2009b). According to our preliminary study (Hong and Chin, 2009), TG activation for cold-set MP gelation was highly correlated to salt and substrate (SC) concentrations, and TG combined with SC was suggested as a best MP gelling system (Kuraishi *et al.*, 1997). In consequence, the efficacy of the TG system on cold-set MP gelation predominantly increased with increasing salt concentration, and the optimum ratio of TG and SC was 6:5 (Hong and Chin, 2009).

CA system, which is one of main cold-set gelling systems, consisted of sodium alginate (SA), calcium carbonate (CC) as a Ca²⁺ source and glucono- δ -lactone (GdL) as an acidulant. Although, calcium chloride was generally used as a basic calcium source in calcium alginate system, this system could not produce a uniform cold-set gel

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due to immediate formation of CA (Means and Schmidt, 1986). Despite of their non-toxic and highly functional properties, application of CA system on cold-set MP gelation was not used commercially. To manufacture low salt, cold-set meat products, CA is the best system, because it can form a thermally irreversible gel at no or low salt concentrations (Means and Schmidt, 1986, 1987; Boles and Shand, 1998; Hong and Chin, 2010b). However, increasing salt concentration decreased the CA gel formation (Hong and Chin, 2010b) because of competition between Na^+ and Ca^{2+} at the G-block binding sites in sodium alginate (Draget *et al.*, 1991). Although, increasing GdL levels in CA system enabled the formation of a cold-set MP gel even with a higher salt concentration, GdL was detrimental in protein-water interactions, resulting in low final yield after thermal processing (Hong and Chin, 2010b). To eliminate GdL induced moisture loss of the MP gel, all systematic components i.e., SA, CC and GdL, had to be optimized at various salt concentrations, and the results showed that optimum ratio of SA, CC and GdL levels was 1:0.3:1 (Hong and Chin, 2009).

Consequently, MP containing relatively no (< 0.1 M) or high salt (> 0.3 M) enabled the formation of a cold-set gel by addition of CA or TG systems, while these systems had no effects on MP gelation at approximately 0.2 M salt concentration which was a typical salt content in actual meat products (Hong and Chin, 2009). Based on these results, a stable cold-set MP gel could be formed at salt concentrations between 0.1 and 0.4 M by combining these two cold-set gelling systems. Therefore, this study aimed to find out the best combination of TG with CA system at various salt concentrations and to determine the changes in the interactions involved in MP gelation.

Materials and Methods

Materials

Porcine *longissimus dorsi* muscles (crossbreed of Landrace×Yorkshire×Duroc, 6 month old hogs), with pH 5.5–5.7, were selected from three carcasses at 24 h post-mortem. The pork loin trimmed off all visible fat and connective tissue and cut into 1 cm cubes. The meat cubes were distributed into 200 g portions, vacuum-packed in a polyethylene pouch, and frozen at -70°C prior to use. Microbial transglutaminase (TG; Activa-TI, 1% TG with 99% maltodextrin) was donated by Ajinomoto Food Ingredients (Chicago, IL, USA). Sodium alginate (SA) with 230 kDa of molecular weight and 0.38 of M/G ratio (ratio of mannuronic to galuronic acid) was purchased from

Junsei Chemical (Tokyo, Japan). All other chemicals were analytical grade.

Extraction of Myofibrillar Protein (MP)

Frozen meat was thawed at 4°C for 12 h, and MP was extracted as previously published by Xiong (1993) with minor modifications (Chin *et al.*, 2009a). In brief, 200 g of minced meat was washed three times with 800 mL of 0.1 M NaCl, 50 mM sodium phosphate buffer (pH 6.25), then the resulting myofibrillar solution was filtered using two sheets of cheesecloth and adjusted to pH 6.25. Finally, the solution was washed with 1.6 L of 0.1 M NaCl solution (pH 6.25). Centrifugation was carried out at $3,000\times g$ for 15 min at 4°C using an auto-refrigerated centrifuge (J2-21, Beckman Inc, USA). After washing the muscle sample, the MP concentration of pellet was determined by the Biuret method (Gornall *et al.*, 1949).

Sample Preparation

The MP solutions were formulated as shown in Table 1. In this study, the TG system consisted of TG and sodium caseinate (SC) at a ratio of 6:5 (w/w), and the CA system with SA, calcium carbonate (CC) and glucono- δ -lactone (GdL) at 1:0.3:1 (w/w), since these ratios were found as the best combination according to our preliminary study (Hong and Chin, 2009). The concentrations of each system indicated those of main components, e.g., 0.5 wt% CA system consisted of 0.5 wt% SA, 0.15 wt% CC and 0.5 wt% GdL. All ingredients were individually dissolved in 50 mM sodium phosphate buffer (pH 6.25) containing targeted salt concentrations from 0.1 to 0.4 M. TG, CC and GdL solutions were prepared just prior to mixing all ingredients. The MP solutions were centrifuged at $750\times g$ for 30 s at 4°C to remove air cells. For analysis, 5 g aliquots of samples were loaded into glass tubes with 12 mm diameter, and incubated at 4°C for 24 h.

pH

The pH of the MP gels incubated at 4°C for 24 h was measured using a pH meter (MP 120, Mettler-Toledo GmbH, Küssnacht, Switzerland) by inserting an electrode into the gels.

Protein Fractionation

Proteins were fractionated by the method of Fernández-Martín *et al.* (2002). Five grams of MP gel stored at 4°C for 24 h was homogenized using a homogenizer (SMT Co. Ltd., Tokyo, Japan) at 2°C for 1 min with 20 mL of a 0.6 M NaCl solution, then centrifuged at $10,000\times g$ for 30

Table 1. Experimental design and responses from seven dependent variables

Treatments	Independent variables			Dependent variables (n=9)						
	Salt (M)	TG system (wt%) ^a	CA system (wt%) ^b	pH	Protein solubility (%)			Gel strength (g)		Yield (%)
	X ₁	X ₂	X ₃		S1	S2	S3	Cold-set	Heated	
1	0.25 (0)	0.60 (0)	0.60 (0)	5.46	25.36	30.55	44.10	63.2	69.0	97
2	0.25 (0)	0.60 (0)	0.00 (-1.4)	6.24	9.21	48.40	42.39	14.3	33.4	78
3	0.25 (0)	0.60 (0)	0.60 (0)	5.46	26.30	32.73	40.96	65.3	70.1	99
4	0.25 (0)	1.20 (1.4)	0.60 (0)	5.45	20.70	21.96	57.34	113.7	151.9	100
5	0.40 (1.4)	0.60 (0)	0.60 (0)	5.45	14.25	28.94	56.81	118.2	208.9	100
6	0.36 (1)	1.02 (1)	0.18 (-1)	5.98	9.37	25.11	65.52	253.3	310.2	96
7	0.14 (-1)	1.02 (1)	1.02 (1)	5.33	18.36	30.48	51.16	494.8	307.5	93
8	0.36 (1)	0.18 (-1)	1.02 (1)	5.22	28.00	44.82	27.18	110.6	134.4	98
9	0.25 (0)	0.60 (0)	0.60 (0)	5.49	22.70	33.24	44.06	66.9	67.1	98
10	0.25 (0)	0.60 (0)	1.20 (1.4)	5.25	18.46	45.41	36.13	280.9	161.6	96
11	0.14 (-1)	0.18 (-1)	1.02 (1)	5.32	25.87	57.06	17.07	497.4	279.6	93
12	0.25 (0)	0.60 (0)	0.60 (0)	5.50	29.94	30.91	39.15	65.5	71.8	99
13	0.10 (-1.4)	0.60 (0)	0.60 (0)	5.53	22.10	37.29	40.60	252.2	130.5	89
14	0.36 (1)	1.02 (1)	1.02 (1)	5.29	23.45	25.39	51.16	174.3	205.8	100
15	0.18 (-1)	0.18 (-1)	0.18 (-1)	5.97	8.73	60.30	30.97	6.5	20.6	88
16	0.25 (0)	0.60 (0)	0.60 (0)	5.54	24.57	33.59	41.84	63.2	68.5	97
17	0.25 (0)	0.00 (-1.4)	0.60 (0)	5.43	44.44	40.92	14.64	39.7	29.2	98
18	0.25 (0)	0.60 (0)	0.60 (0)	5.53	27.50	32.70	39.80	64.3	69.8	98
19	0.18 (-1)	1.02 (1)	0.18 (-1)	5.92	15.90	33.19	50.91	15.5	35.8	82
20	0.36 (1)	0.18 (-1)	0.18 (-1)	5.96	12.88	59.46	27.67	25.6	177.6	100

^aThe value presents transglutaminase (TG) concentration among TG system consisted with TG and sodium caseinate as a ratio of 6:5.

^bThe value presents sodium alginate (SA) concentration among calcium alginate (CA) system consisted of SA, calcium carbonate and glucono- δ -lactone as a ratio of 1:0.3:1.

min at 4°C. This procedure was repeated once, and the two supernatants were mixed and expressed as S1 (salt-soluble) fraction. The remaining precipitate was homogenized at 2°C for 1 min with 20 mL of 4 M urea buffer containing 0.3 M NaCl, stored at 4°C for 24 h, and then centrifuged twice with the same condition as before (S1). The combination of the two supernatants was expressed as S2 (urea-soluble) fraction. Protein concentrations (%) of S1 and S2 fractions were determined by the Biuret method (Gornall *et al.*, 1949). The insoluble fraction (S3) was numerically calculated by difference in percentages of total (100%) and soluble fraction (sum of S1 and S2). According to Kauzmann (1959), S1 fraction is composed of proteins remaining in a native-like conformation and intermolecularly associated by electrostatic bonds, S2 is aggregated intermolecularly linked by hydrophobic interactions and hydrogen bonds, and S3 is protein aggregates formed by covalent, disulfide bonds and other insoluble proteins (Fernández-Martín *et al.*, 2002).

Gel Strength

Gel strengths of cold-set MP gels after incubation at 4°C for 24 h were determined using an Instron universal

testing machine (3340, Instron Corporation, Canton, MA, USA) equipped with a 9 mm diameter stainless steel plunger and cross-head speed 50 mm/min. The plunger was penetrated 1 cm into the MP gel of which dimensions were 12 mm diameter and 20 mm height. Maximum forces (g) were taken and estimated as gel strengths of cold-set MP gels.

To evaluate the changes in gel strengths of cold-set MP gels after thermal treatment, the gels were heated from 0 to 75°C at 3°C/min using a programmable water bath. After thermal treatment, samples were immediately cooled in an ice for 10 min and tempered in ambient for 4 h. The measurement was performed with the same method as the cold-set gels.

Cooking Yield

The cooking yields were measured to evaluate the water binding properties of MP gels. The samples were thermal treated as described in previous section, and the liquid exudates were discarded. Cooking yields were determined by assessing the weight of gels, and expressed as a percentage of initial weight.

Statistical Analysis

Response surface methodology was adapted to determine relationships between NaCl concentrations and MP gelling systems. Five levels of NaCl, TG system and CA system concentrations were selected based on central composite design (Table 1). The complete design composed of 20 groups with six replications of the center point to estimate pure error (Cochran and Cox, 1992). The models were expressed by a second-order polynomial equations as:

$$Y = \beta_0 + \sum_i^k \beta_i X_i + \sum_i^k \beta_{ii} X_i^2 + \sum_{i < j}^k \beta_{ij} X_i X_j$$

where Y is the estimated response, β_0 , β_i , β_{ii} and β_{ij} are regression coefficients, X_i and X_j are the independent variables, and k is the number of independent variables. The significances of all terms in the polynomial were judged using SAS 9.1 (SAS Institute, Inc., Cary, NC) by computing the F-value at a probability (p) of 0.001, 0.01 or 0.05 as described by Lee *et al.* (2006).

Results and Discussion

pH

The pH values of MP gels ranged from 5.4 to 6.2, and

inversely proportional to CA level regardless of salt or TG concentrations (data were not shown). In the regression model (Table 2), only the CA system affected the pH of MP gels, having both linear and quadratic terms ($p < 0.001$). Salt had an interaction effect with the CA system ($p < 0.05$), while the TG system had no effects on the pH of MP gels ($p > 0.05$). It is generally recognized that the activation of both CA and TG systems depends on the pH (Sakamoto *et al.*, 1994; Mancini *et al.*, 1999). For the CA system, GdL has the potential to slowly reduce the pH via acid hydrolysis (Ngapo *et al.*, 1996), which results in the slow release of Ca^{2+} from CC (Draget *et al.*, 1991). Although CC increased the pH with increasing incubation time, the degree of increment in pH was negligible when compared to the effect from GdL (Hong and Chin, 2009a). On the other hand, the initial pH of all components was adjusted to 6.25, resulting in no effects of salt or the TG system on the final pH of MP gels.

Protein Fractionation

Increasing TG system tended to decrease the salt-soluble (S1) fraction of MP, while, the S1 fraction increased with increasing CA system up to 0.7% following decreased (Fig. 1). The CA system affected the S1 fraction, being

Table 2. Regression coefficients and analysis of variances of regression models

Term	pH	Protein solubility			Gel strength		Yield
		S1	S2	S3	Cold-set	Hot-set	
β_0	6.28	-8.7	81.2	28.3	170.2	131.3	68.8
Linear							
β_1	-0.42	168.0*	-54.1	-120.0	-2483.6***	-1839.8***	102.3*
β_2	-0.03	-11.4	-38.0*	49.9**	-151	-185.2*	-17.9
β_3	-1.61***	53.3**	-59.4***	3.3	667.0***	402.6***	44.2***
Quadratic							
β_{11}	0.68	-351.3*	79.2	287.6	6225.7***	5868.9***	-84.4
β_{22}	-0.08	13.8	4.3	-17.3	87.7	147.0*	7.9
β_{33}	0.75***	-34.0***	43.3***	-8.3	284.6***	166.3**	-25.4***
Crossproduct							
β_{12}	0.41	-22.8	-4.8	21.7	791.6**	446.8*	11.1
β_{13}	-0.55*	28.1	-18.8	-3.3	-2678.6***	-1884.3***	-38.9
β_{23}	0.07	-11.8	11.8	0.2	-121.8	-33.7	7.6
R^2	0.97	0.50	0.73	0.78	0.87	0.80	0.63
F-value							
Lack-of-fit	0.58	2.09	1.44	0.91	1.34	1.15	2.07
Factor analysis ^a							
Salt	2.43	1.59	1.44	2.33	48.10***	36.96***	10.25***
TG system	1.48	1.93	18.1***	23.78***	7.26***	8.73***	1.17
CA system	377.02***	7.36***	6.95***	1.2	72.23***	34.39***	12.02***

^aTG, transglutaminase; CA, calcium alginate.

*Significant at 0.05 levels.

**Significant at 0.01 levels.

***Significant at 0.001 levels.

Fig. 1. Contour plots for salt-soluble fraction (S1) in myofibrillar proteins (MP) as a function of transglutaminase and sodium alginate at (a) 0.1 M and (b) 0.4 M NaCl levels, respectively. The numbers on contour lines indicate the concentration of salt-soluble protein (%) of MP.

linear ($p < 0.01$) and quadratic ($p < 0.001$) terms. In the regression model, salt also had linear and quadratic effects ($p < 0.05$) on the S1 fraction, but no significance in factor analysis was found (Table 2). Meanwhile, the TG system had no influence on the S1 fraction which mainly consisted of proteins native-like or intermolecularly associated via electrostatic bonds (Fernández-Martín *et al.*, 2002; Moreno *et al.*, 2008). Not only was divalent Ca^{2+} much more effective at screening electrostatic repulsion and at binding to charged sites on MPs than monovalent Na^+ (Kulmyrzaev *et al.*, 2000), but as Ca^{2+} simultaneously bound to two negatively charged surface on MPs (Bryant and McClements, 1998), the CA system might be more significant than salt in factor analysis. However, electrostatic interactions were very sensible to pH, and SA was more sensible to Ca^{2+} than MPs. Furthermore, the bond strength of electrostatic interactions was not highly

enough to form a MP gel (Park, 2000), so the impact of Ca^{2+} on electrostatic interactions in MPs was possibly lower than other interactions (S2 and S3 fractions).

The urea-soluble (S2) fraction, which mainly composed of aggregates intermolecularly linked by hydrophobic interactions and hydrogen bonds (Fernández-Martín *et al.*, 2002; Moreno *et al.*, 2008), was reversely proportional to TG system (Fig. 2). The CA system decreased the S2 fraction with increasing its concentration up to ca. 0.7%. Both TG ($p < 0.05$) and CA ($p < 0.001$) systems had linear effects on the S2 fraction. In addition, the CA system affected this fraction by quadratic manner ($p < 0.001$). In factor analysis (Table 2), salt did not affect the S2 fraction ($p > 0.05$). Although neither hydrophobic interaction nor hydrogen bonds were sensitive to pH (Bryant and McClements, 1998), it seemed that there were involved some complicated factors in CA system. At low CA sys-

Fig. 2. Contour plots for urea-soluble fraction (S2) in myofibrillar proteins (MP) as a function of transglutaminase and sodium alginate at (a) 0.1 M and (b) 0.4 M NaCl levels, respectively. The numbers on contour lines indicate the concentration of urea-soluble protein (%) of MP.

Fig. 3. Contour plots for insoluble fraction (S3) in myofibrillar proteins (MP) as a function of transglutaminase and sodium alginate at (a) 0.1 M and (b) 0.4 M NaCl levels, respectively. The numbers on contour lines indicate the concentration of insoluble protein (%) of MP.

tem, the liberated Ca^{2+} might break down the hydrogen bonds within MPs, resulting in lowered S2 fraction. Meanwhile, GdL induced acidification would be dominated at high amount of CA system, and thus increased the S2 fraction of MP. It should be noted that the CA system did not directly interact with MPs (Moreno *et al.*, 2008; Hong and Chin, 2009a) and thermodynamical incompatibility may be present between MP and SA due to a negative net charge at 6.25 unit of initial pH (McClements, 2006). However, GdL induced an acidic condition which might have reduced the exclusive interactions between two biopolymers.

Fig. 3 shows the contour maps for the insoluble (S3) fraction as affected by MP gelling systems. Although the S3 fraction was increased at 0.4 M NaCl, no effect of salt on the S3 fraction was observed both in regression and in factor analysis (Table 2). The CA system did not affect on

the S3 fraction, while, the S3 fraction increased with increasing TG system. For both regression model and factor analysis, TG alone showed a linear effect ($p < 0.01$) on this fraction. Because TG caused cross-linking among MPs, the insoluble fraction, which was mainly covalent bonds (Fernández-Martín *et al.*, 2002), was proportional to TG concentration. Moreover, the covalent bond had high bond strength among interactions (McClements, 1999), and therefore, had a direct correlation with gel strength.

Gel Strength

Gel strengths of cold-set MP incubated at 4°C for 24 h as affected by MP gelling systems are presented in Fig. 4. At 0.1 M NaCl, the gel strength was directly proportional to only CA system, while, the impact of TG system increased with increasing NaCl concentration, especially

Fig. 4. Contour plots for cold-set gel strengths of myofibrillar proteins (MP) as a function of transglutaminase and sodium alginate at (a) 0.1 M and (b) 0.4 M NaCl levels, respectively. The numbers on contour lines indicate the gel strength (g) of MP, and the optimized domain was colored (> 50 g).

at 0.4 M NaCl. Both salt and the CA system influenced the gel strength of cold-set MPs ($p < 0.001$). Salt also had interaction effects with TG ($p < 0.01$) and CA ($p < 0.001$) systems. Meanwhile, the TG system alone had no effects on cold-set MP gel strength in a regression model ($p > 0.05$). Since the TG system had no effects on gelation at 0.1 M NaCl, gel strength was only proportional to CA system. This might be due to CA gel network formation at low NaCl concentration as well as acidification by GdL. Meanwhile, higher the SA concentration at 0.4 M NaCl concentration, lower the gel strength, even though large amount of TG was added to the MP. The results indicated that CA gel lost their gelling characteristics at relatively high salt concentrations as well as inactivating the TG system and MP gel formation.

Since 50 g of gel strength was high enough to be eval-

uated as a cold-set gel (Hong and Chin, 2009a), further increased gel strength (> 50 g) of cold-set MP gels was not necessary for optimization. Meanwhile, the MP gel texture should not be disrupted after thermal treatment, and hence, gel strength of MP had to increase after thermal treatments. Therefore, a high MP gel strength after thermal treatment must be considered as an important gel characteristic, and the results are presented in Fig. 5. Contour maps for gel strength of MP after thermal treatment showed similar pattern with the cold-set gels, but the TG system had a remarkable effect on gel strength at high salt concentration. According to the regression model, all independent variables had linear and quadratic effects on MP gel strength after thermal treatment ($p < 0.05$) with the exception of the interaction between TG and CA systems (Table 2). The increased MP gel strength after thermal

Fig. 5. Contour plots for gel strengths of heat treated myofibrillar proteins (MP) as a function of transglutaminase and sodium alginate at (a) 0.1 M and (b) 0.4 M NaCl levels, respectively. The numbers on contour lines indicate the gel strength (g) of MP, and the optimized domain was colored (> 100 g).

Fig. 6. Contour plots for cooking yield of myofibrillar proteins (MP) as a function of transglutaminase and sodium alginate at (a) 0.1 M and (b) 0.4 M NaCl levels, respectively. The numbers on contour lines indicate the cooking yield (%) of MP, and the optimized domain was colored ($> 95\%$).

processing was mainly due to the optimum activating condition for TG at 50°C and pH 9 when SC was used as a substrate (Sakamoto *et al.*, 1994). Consequently, the results showed that both TG and CA systems contributed to MP gel strength by balancing their efficacy under various salt concentrations and thermal treatment. The CA system governed gel strength of cold-set MP gels at low NaCl concentration, whereas MP gel strength after thermal treatment was attributed to TG at high NaCl concentration. In the two phase system composed of MPs and anionic polysaccharides, it would be useful to have a strategic approach such as in-situ gelation (Neiser *et al.*, 1999), by which the phases of both MP and CA transformed from solution to viscoelastic gel without phase separation. At a moderate salt concentration as 0.25 M NaCl, a common concentration in muscle foods, viscoelastic gel could only be produced by combining the two systems. From the in-situ gelation point of view, the combination of the two systems had a potential benefit because MP gels had not only high gel strength at a cold-set state, but also improved gel strength after thermal treatment with containing high amount of moisture.

Cooking Yield

Contour maps for the cooking yield of MP gels as a function of TG and SA levels are shown in Fig. 6. Increasing CA system improved the cooking yield of MP, while the cooking yield was decreased at extreme amount of CA system. Moreover, the cooking yield increased with increasing NaCl concentration. Salt concentrations had a positive effect on the yield of MP gels ($p < 0.05$). In addition, the CA system had linear and quadratic terms ($p < 0.001$) on the cooking yield of MP. However, no effect of the TG system on cooking yield was found ($p > 0.05$). According to our previous study (Hong and Chin, 2009a), the CA system improved the water holding capacity of cold-set MP gel after thermal treatment, but the addition of a high amount of GdL with a corresponding CA system concentration in this study showed negative effects on yields, mainly due to pH decline. On the other hand, no effect of TG system on yield of MP gel in this study was different from our previous finding, where TG system reduced the cooking yield of MP gel (Hong and Chin, 2010a). The TG system enhanced protein-protein interactions in MPs, hence it may result in high moisture loss during thermal processing (Chin *et al.*, 2009a). However, increasing SC concentration with increasing TG in the current study might minimize the TG induced moisture loss in MP gel. In conclusion, the TG system contrib-

uted to textural properties of MPs, particularly gel strength of MP after thermal treatment, while the CA system governed gel texture of cold-set state and water-binding property, and an acceptable viscoelastic MP gel with high moistness could only be formed by combining these two systems.

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