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Research Article

Effects of Sodium Tripolyphosphate, Microbial Transglutaminase and Enzyme-hydrolyzed Soy Protein Fraction on the Quality of Cooked Pork Batter by Response Surface Methodology

[1, 2]Xingjian Huang, [1, 3]Tian Yi, [4, 5]Fang Yang, [3]Can Xu, [3]Gan Li, [1, 3]Wanfeng Hu,
[2]Dingren Bi and [1, 3]Siyi Pan
[1]MOE Key Laboratory of Environment Correlative Dietology,
[2]College of Animal Science and Technology,
[3]College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, P.R. China
[4]Department of Animal Sciences, Food Science Section, University of Kentucky, Lexington, Kentucky 40546, United States
[5]Department of Biological Engineering, Zhixing College of Hubei University, Wuhan, Hubei 430011, P.R. China

Abstract: We investigated the compound effects of sodium tripolyphosphate (STPP), microbial transglutaminase (MTGase) and enzyme-hydrolyzed soy protein fraction (denoted as TSF, molecular weight cut-off = 0.5 kDa to 10 kDa) on the texture properties (hardness, springiness, cohesiveness and chewiness), cooking yield and sensory attributes (firmness, elasticity and juiciness) of cooked pork batter. The hardness and springiness of the cooked pork batter were both significantly affected by the amount of MTGase and TSF added. In the presence of TSF, the textural characteristics of cooked pork batter were not significantly affected by STPP ($p>0.05$). The amount of TSF elicited negative linear ($p<0.001$) and positive quadratic effects ($p<0.01$) on the cohesiveness and chewiness of cooked pork batter. The interaction between MTGase and TSF positively affected ($p<0.01$) the cohesiveness of cooked pork batter. Furthermore, the amount of MTGase showed positive linear ($p<0.01$) effects on the chewiness of cooked pork batter. However, the interaction between STPP and TSF significantly weakened ($p<0.05$) the chewiness of cooked pork batter. Both TSF and MTGase positively affected ($p<0.01$ and $p<0.05$, respectively) cooking yield. Both hardness versus firmness and springiness versus elasticity presented distinct correlations ($p<0.01$ and $p<0.001$, respectively). The cohesiveness and chewiness of cooked pork batter significantly affected cooking yield and sensory attributes (firmness, elasticity and juiciness). Overall acceptability poorly correlated with instrumental attributes and sensory partial attribute. Sensory analysis results indicated that the cooked pork batter with 0.4% MTGase, 4% TSF and 0.4% STPP was the most common sample, which presented the best synthetic mouth feeling.

Keywords: Microbial transglutaminase, pork batter, soy protein, sensory, texture, ultrafiltration

INTRODUCTION

Sodium chloride, sodium phosphates, sodium lactate, polysaccharide gums, hydrolyzed soy or whey proteins and modified starch can be added to meat products and have been reported to satisfy functional needs (Xiong, 2005). Pyrophosphate, tripolyphosphate, or metaphosphates are the common phosphates used in injected meats. Among these phosphates, Sodium Tripolyphosphate (STPP) strongly increases electrostatic interactions and reduces hydrophobic interactions involved in protein sol-matrix formation (Fernández-Martín *et al*., 2002). The presence of these highly charged individual or mixed compounds enables the injected meat to effectively retain water, thereby improving cooking yield and textural palatability (Hamm, 1986). Numerous non-meat proteins have been investigated to determine their effects on textural properties of complex muscle foods, such as frankfurters and batters. Non-meat proteins can directly interact with meat proteins and occupy the interstitial spaces in a gel matrix or gel as discrete pockets in a matrix structure (Pietrasika *et al*., 2007). Added microbial transglutaminase (MTGase) does not contribute (or enhance/reduce) to the effect of salt on water-binding properties of muscle-based products

Corresponding Author: Wanfeng Hu, MOE Key Laboratory of Environment Correlative Dietology, Huazhong Agricultural University, Wuhan, Hubei 430070, P.R. China, Tel.: +86-27-87283778; Fax: +86-27-87288373
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(Cofrades *et al*., 2006). The differences in the effects of MTGase on water-binding properties possibly depend on the level and type of MTGase and the conditions in which this enzyme is used, such as reaction temperature and time, meat particle size, disruption methods, presence of other ingredients and meat source. Furthermore, a wide range of vegetable proteins can be cross-linked by MTGase. Some of these proteins, including globulin and gluten, either on their own or as part of a meat extender, provide a good substrate for MTGase reaction; as a result, polymers are formed and such polymers significantly improve the technological properties of comminuted meat (Colmenero *et al*., 2005).

Hydrolysis can help soy protein improve the gelling properties of Myofibrillar protein and the water retention ability of mixed protein gel. Feng and Xiong (2003) reported that the partial substitution of native Soy Protein Isolate (SPI) for myofibrillar protein likely impairs the dynamic storage modulus of myofibrillar protein gels; however, SPI hydrolysate alleviates adverse effects. Enzyme-hydrolyzed soy protein fraction [molecular weight cut-off (MWCO) < 10 kDa] exhibits higher solubility and molecule flexibility than other soy proteins, thereby facilitating interaction with myofibrillar protein (Guillén *et al*., 1997; Huang *et al*., 2010). Kuraishi *et al*. (1997) further reported the negative effect of high amounts of MTGase on gel strength and water holding capacity. They proposed that excessive MTGase and (γ-Glu) Lys bonds possibly inhibit the uniform development of a protein network to entrain sufficient water (Carrascal and Regenstein, 2002).

Texture and water holding capacity are properties that significantly influence palatability and consumer acceptance of foods (Jeremiah *et al*., 1999). Different ingredients in foods may be replaced or reduced, but this process usually changes these properties of food gels (Tseng *et al*., 2000a). Protein hydrolysates can contribute to water holding capacity because of the strong hydrophilicity of soy peptides and possibly their synergistic interactions with muscle proteins, forming a gel matrix that can immobilize extraneous water (Feng and Xiong, 2002). However, the exact function of hydrolyzed soy proteins in the gel formation of muscle proteins and their effect on the quality of the composite gel system and comminuted meat products have not been clearly elucidated. Although the physical characteristics of meat are usually assessed instrumentally, meat texture characteristics are useless if they are not supported by sensory evaluation; this finding shows that sensory evaluation can provide information related to experiences during meat consumption. Thus, descriptive-quantitative analysis should be conducted using a trained panel; texture profile should also be determined (Huidobro *et al*., 2005).

The present study aimed to evaluate the composite effects of STPP, MTGase and enzyme-hydrolyzed soy protein on the texture properties (hardness, springiness, cohesiveness and chewiness), cooking yield and sensory attributes (firmness, elasticity, juiciness and overall acceptability) of cooked pork batter by response surface methodology. Correlations between hardness, springiness, cohesiveness, chewiness, cooking yield, firmness, elasticity, juiciness and overall acceptability were obtained and statistically evaluated by Pearson correlation test.

MATERIALS AND METHODS

Enzyme hydrolysis of soy proteins: Hydrolysis was performed with partial denaturation (90°C, 5 min). Preheated SPI solutions (5% protein, pH 8.85; Shandong Yuwang Industrial Co., Ltd., Jinan, Shandong, China) were hydrolyzed at 40°C by trypsin (1:250; Amresco Inc., Solon, OH, USA) for 4 h. Withdrawn solutions were immediately heated at 90°C for 10 min to inactivate enzymes and cooled to room temperature. Precipitates were removed by centrifugation (3,000×*g*) and supernatants were collected.

Peptide fractionation by ultra filtration: Hydrolysate supernatants were further fractionated by ultrafiltration with an Amicon stirred cell and disk membrane system (Model 8400; volume size of 400 mL). Protein hydrolysate supernatant was adjusted to pH 7.0 and initially separated by a 100 kDa MWCO membrane (Spectrum Medical Industries Inc., Houston, TX, USA) from the cell at 70 psi and 4°C. The separations generated two streams, namely, retentate and permeate 1. Permeate 1 was further separated using the 10 kDa MWCO membrane (Spectrum Medical Industries Inc., Houston, TX, USA) and generated two other streams, namely, permeate 2 and retentate 1. Permeate 2 was placed in a Spectra/Por membrane tubing (MWCO = 0.5 kDa; Spectrum Medical Industries Inc., Houston, TX, USA) and dialyzed against 2 L of reverse osmosis-purified water at 4°C. Water was changed every 2 h for the first 6 h and every 12 h thereafter. Permeate 2 was freeze-dried, finely ground in a grinder, sealed in glass bottles and stored at 4°C until analysis (denoted as TSF, MWCO = 0.5 kDa to 10 kDa).

Preparation of cooked pork batter: Fresh pork hindquarters were obtained from a local meat retailer. Proximate analyses of the raw meat were determined in triplicate according to the AOAC methods (AOAC, 2011). The average moisture, protein, fat and ash contents were 67.6, 29.7, 1.8 and 0.9 g/100 g, respectively. Before processing, we tempered the meat at 4°C for 24 h before use (Pietrasik and Li-Chan, 2002). The gross weight of ground meat (lean meat: fat meat, 4:1) and non-meat ingredients was adjusted to 50 g. After visible fat and connective tissue were

Table 1: Central composite design arrangement and experimental result of the response variables of cooked pork batter

	Variable levels			Responses		
Trial no.	X_1	X_2	X_3	Hardness (N)	Springiness	Cohesiveness
1	-1	-1	0	1.87	0.31	0.54
2	-1	1	0	1.18	0.26	0.28
3	1	-1	0	2.24	0.36	0.45
4	1	1	0	1.45	0.31	0.35
5	0	-1	-1	1.78	0.38	0.49
6	0	-1	1	2.16	0.39	0.46
7	0	1	-1	1.48	0.33	0.32
8	0	1	1	1.17	0.26	0.30
9	-1	0	-1	1.62	0.29	0.36
10	1	0	-1	1.67	0.36	0.37
11	-1	0	1	1.28	0.30	0.34
12	1	0	1	1.58	0.37	0.36
13	0	0	0	1.70	0.32	0.39
14	0	0	0	1.63	0.31	0.35
15	0	0	0	1.62	0.31	0.35

Trial no.	Chewiness	Cooking yield (%)	Firmness (panel)	Elasticity (panel)	Juiciness (panel)	Overall acceptability (panel)
1	0.31	81.180	3.95	3.32	2.50	5.0
2	0.08	90.540	2.80	2.90	2.60	3.7
3	0.36	89.100	4.25	3.35	2.61	7.0
4	0.15	98.800	3.70	3.32	2.71	4.0
5	0.33	80.780	3.90	3.50	2.49	4.8
6	0.39	83.640	4.25	3.55	2.50	6.0
7	0.15	98.380	3.74	3.33	2.71	5.8
8	0.09	99.840	2.91	3.05	2.72	7.9
9	0.17	88.160	3.80	3.25	2.55	6.2
10	0.22	93.760	3.81	3.30	2.70	5.7
11	0.13	90.280	2.90	3.27	2.60	7.1
12	0.21	93.660	3.80	3.43	2.70	7.4
13	0.21	102.54	3.81	3.38	2.72	6.9
14	0.17	99.340	3.83	3.34	2.71	6.8
15	0.17	101.78	3.80	3.30	2.72	7.0

X_1 represents MTGase, X_2 represents TSF and X_3 represents STPP. Data are means from three replications

removed, treatments (300 g each) were prepared by mixing ground meat and non-meat ingredients for 15s in Büchi Mixer B-400 (8000 rev/min). The additive levels of STPP (AR, China National Medicines Co., Ltd., China), MTGase (approximately 70 U/g; Cino-Chem Science and Technology Co., Ltd.) and enzyme-hydrolyzed soy protein fraction were determined using a three-level factorial design (Table 1) and described in the experimental design and statistical analysis sections. The final temperature of the homogenates was not more than 12°C.

Immediately after homogenate was prepared, the batters were placed in three cylindrical plastic tubes (30×115 mm) and centrifuged (Model 225; Fisher Scientific, Pittsburgh, USA) at a slow speed (2500×g) to remove all air bubbles. The tubes were closed and allowed to stand for 2 h in a warm water bath at 45°C. The pork batters were cooked in a water bath from 10 to 75°C for 1.5 h and then cooled down in ice water until a core temperature of 20°C was reached. Internal temperature was measured using thermocouples inserted in the geometrical center of the samples (Somboonpanyakula *et al*., 2007). The pork batter samples were stored at 4°C for 12 h before analysis.

Cooking yield: Each chilled gel was stored overnight; afterward, this gel was removed from the porcelain vessel, blotted dry with a paper towel and weighed to determine cooking yield. The weight of the pork batter before and after cooking was measured. Cooking yield was calculated as follows:

$$\text{Cooking yield} = \frac{\text{g batter after cooking} \times 100}{\text{g batter before cooking}}$$

Experimental design: Response surface methodology was performed to investigate the simultaneous effect of the three experimental variables on gel/emulsion system properties. The experiments were based on a Box-Behnken design (Nagarajan and Natarajan, 1999). Three levels of each factor (variable) were selected in accordance with the principles of a central composite design (Table 1). The influential factors were MTGase (0 to 0.8%), TSF (0 to 4%) and STPP (0 to 0.4%) contents. In each experiment, 15 combinations of three variables were performed following the design. Error assessment was based on the replication of the central point treatment combination, as suggested in the Box-Behnken design. For each response, a second-order polynomial equation was fitted as follows:

Table 2: Class definition and reference foods used to assess sensory parameters in meat

Parameter	Standard scale	Class (definition)	Reference food	Non-structured scale (10 cm long)
Firmness	1	Very soft	Philadelphia cheese	0.00-1.11
	2	Soft	Soft cheese	1.12-2.22
	3	Slightly soft	Frankfurter sausage	2.23-3.33
	4	Little firm	Semi-hard cheese	3.34-4.44
	5	Firm	Olive	4.45-5.55
	6	Very firm	Cashew nut	5.56-6.66
	7	Slightly hard	Toasted almond	6.67-7.77
	8	Hard	Fried almond	7.78-8.88
	9	Very hard	Candy sugar	8.89-10.0
Elasticity	1	Non-elastic (or plastic)	Margarine	0.0-2.0
	2	Slightly springy	Edam cheese	2.1-4.0
	3	Springy	Marshmallows	4.1-6.0
	4	Quite springy	Squid	6.1-8.0
	5	Extremely springy	Gominola	8.1-10.0
Juiciness	1	Dry	Biscotte	0.0-2.0
	2	Moist	Banana	2.1-4.0
	3	Wet	Apple	4.1-6.0
	4	Watery	Orange	6.1-8.0
	5	Juicy	Water melon	8.1-10.0

$$y = b_0 + \sum_{i=1}^{k} b_i x_i + \sum_{i=1}^{k} b_{ii} x_i^2 + \sum_{i<k}^{k} b_{ij} x_i x_j$$

where, y is the estimated response and b_0, b_i, b_{ii} and b_{ij} are the equation parameter estimates (constant, b_0; parameter estimates for linear terms, b_i; for quadratic terms, b_{ii}; for interaction terms, b_{ij}), x_i and x_j are the factor levels and k is the number of factors. The significance of the equation parameters for each response variable was assessed by F test using Statistical Analysis Systems Ver. 9.1.3.; (SAS, 2004). Several response surfaces were obtained to determine the main effects of MTGase, TSF and STPP levels on the dependent experimental parameters. These 3D representations show the effect of two given independent variables in a particular response, imposing a constant value equal to the mid-level of the third variable.

Textural properties: The textural characteristics of gels were analyzed according to the Texture Profile Analysis (TPA) method by using a texture analyzer (Model TA-XT2; Stable Micro System, Surrey, England) equipped with aluminum cylinder probe (diameter = 36 mm) with a radiused edge (P/36R). The sample was placed under the probe that moved downward at a constant speed of 3.0/mms (pre-test), 1.0/mms (test) and 3.0/mms (post-test). The thickness of the sample was automatically recorded by the Texture Exponent 32 software when the probe initially came in contact with the sample. The probe continued downward to a pre-fixed percentage of the sample thickness (75%), returned to the initial point of contact with the sample and stopped for a set period of time (2 s) before the second compression cycle was initiated. During the test run, the resistance of the sample was recorded at an interval of 0.01 s and plotted in a force-time (grams-seconds) plot, as illustrated schematically by Veland and Torrissen (1999). A constant compression speed was used in all of the tests (1.0/mms); the areas under the force-time curve were directly proportional to the test performed by the probe and sample during downstroke and upstroke, respectively. In the force-time plot, the TPA parameters were read or calculated as follows: hardness (peak force on first compression, N); cohesiveness (ratio of the active work performed under the second force-displacement curve to that performed under the first compression curve, dimensionless); springiness (distance of the sample recovered after the first compression, dimensionless); and chewiness (hardness× cohesiveness× springiness, dimensionless) (Ta´rrega and Costell, 2007).

Sensory analysis: Sensory evaluations were conducted by a trained 10-member panel (five males and five females; mean age = 35 years; range = 18 years to 54 years) in an environmentally controlled (21±1°C; relative humidity of 55±5%) room partitioned into booths (Giuseppe *et al*., 2012; ISO-8586-2012, 2012). The samples were reheated in a microwave oven for 30 s and served at 45±2°C. Each sample was coded with randomly selected three-digit numbers.

Panelists recorded descriptive sensory evaluations. Onega and Ruiz de Huidobro (Ruiz de Huidobro *et al*., 2001) used reference scales to assess the intensity of parameters and proposed some food as standards for scale points (Table 2). Sensory attributes included firmness (1 = very soft, 10 = very firm), elasticity (1 = low elasticity, 10 = high elasticity) and juiciness (1 = very dry, 10 = very juicy). The samples were served randomly and evaluated in two replicates by all of the panelists. The panelists were also instructed to clean their palates with water before they tasted another sample. Each attribute was discussed and tests were initiated after the panelists were familiarized with the scales. At the end of the test, the panelists were instructed to provide a score (from 0 to 10) of the overall acceptability of each sample (Melendres *et al*., 2014).

Correlation coefficient analysis: We performed Pearson correlation test to evaluate correlations. The correlation coefficients and associated probability values were calculated and significance level was set at $p<0.05$ unless otherwise stated. Statistical Analysis Systems Ver. 9.1.3.; (SAS, 2004) was used to analyze the data.

RESULTS AND DISCUSSION

Model fitting from RSM for TPA and cooking yield: Table 1 shows the effects of MTGase, enzyme-hydrolyzed soy protein fraction (TSF, MWCO = 0.5 kDa to 10 kDa) and STPP on hardness, springiness, cohesiveness, chewiness and yield of cooked pork batter. The independent and dependent variables were fitted to the second-order model equation and examined for the goodness-of-fit.

ANOVA was performed to determine the lack-of-fit and the significance of the linear, quadratic and interaction effects of independent variables on dependent variables (Table 3). The lack-of-fit test is a measure of the failure of a model to represent data in the experimental domain, in which points were not included in the regression. The coefficient of determination or R^2 is the proportion of variation in the response attributed to the model rather than random error and suggested that R^2 should be at least 80% for a good fit model (Sobhi *et al*., 2012). The results showed that the models of all the response variables were highly adequate because they yield satisfactory levels of R^2 (>80%) and no significant lack-of-fit was observed in all of the response variables.

Regression coefficients are shown in Table 4 and the equations of each of the response variables can be derived from the predicted values of each response variable. R^2 of the responses exceeded 80%, indicating a high proportion of variability, as explained by the data. Therefore, the developed response surface models were adequate. The fitted second-order polynomials of the response variables were expressed as follows:

$$\text{Hardness} = 1.651+0.123X_1-0.347X_2-0.044X_3-0.035X_1^2-0.026X_1X_2+0.073X_2^2+0.062X_3X_1-0.173X_3X_2-0.078X_3^2$$

$$\text{Springiness} = 0.314+0.030X_1-0.035X_2-0.004X_3-0.010X_1^2+0.001X_1X_2+0.002X_2^2+0.003X_3X_1-0.020X_3X_2+0.023X_3^2$$

$$\text{Cohesiveness} = 0.358+0.001X_1-0.086X_2\ 0.008X_3+0.005X_1^2+0.038X_1X_2+0.040X_2^2+0.001X_3X_1+0.001X_3X_2-0.007X_3^2$$

$$\text{Chewiness} = 0.187+0.031X_1-0.113X_2-0.005X_3-0.010X_1^2+0.005X_1X_2+0.050X_2^2+0.008X_3X_1-0.032X_3X_2+0.004X_3^2$$

$$\text{CY} = 101.220+3.145X_1+6.608X_2+0.793X_3-5.255X_1^2+0.085X_1X_2-6.060X_2^2-0.555X_3X_1-0.350X_3X_2-4.500X_3^2$$

Table 3: ANOVA showing the linear, quadratic interaction and lack-of-fit of the response variables for texture profile analysis and cooking yield

		Response variables									
		Hardness (N)		Springiness		Cohesiveness(mm)		Chewiness(N×mm)		Cooking yield (%)	
Source of variation	Df	Sequential sum of squares	*F*	Sequential sum of squares	*F*	Sequential sum of squares	*F*	Sequential sum of squares	*F*	Sequential sum of squares	*F*
Regression											
Linear	3	1.100303	37.21***	0.017218	14.59**	0.060169	62.64***	0.109519	129.51***	433.425100	18.11**
Quadratic	3	0.050094	1.69ns	0.002528	2.14ns	0.006201	6.46*	0.009934	11.75*	271.657373	11.35*
Crossproduct	3	0.136878	4.63ns	0.001643	1.39ns	0.005785	6.02*	0.004357	5.15ns	1.751000	0.07ns
Total model	9	1.287275	14.51**	0.021389	6.04*	0.072154	25.04**	0.12381	48.80***	706.833473	9.84*
Lack-of-fit	3	0.045556	8.16ns	0.001823	8.46ns	0.000534	0.33ns	0.000445	0.31 ns	34.299700	4.09ns
R^2 (%)		96.3		91.6		97.8		98.9		94.7	

*: Significant at p≤0.05; **: Significant at p≤0.01; ***: Significant at p≤0.001; ns: not significant; Df = Degree of Freedom; *F* = rate of variance estimates

Table 4: Estimated regression coefficients of the fitted second-order polynomial for the response variables for texture profile analysis and cooking loss

	Estimated coefficients				
Coefficients	Hardness (*N*)	Springiness	Cohesiveness (mm)	Chewiness (*N*×mm)	Cooking yield (%)
Constant	1.650667***	0.314333***	0.358333***	0.186667***	101.220000***
MTGase	0.123125*	0.030225**	0.000625ns	0.031250**	3.145000*
TSF	-0.347000***	-0.034963**	-0.086375***	-0.112625***	6.607500**
STPP	-0.044375ns	-0.004038ns	-0.007750ns	-0.005375ns	0.792500ns
MTGase×MTGase	-0.035458ns	-0.009854ns	0.005083ns	-0.009708ns	-5.255000*
MTGase×TSF	-0.025500ns	0.001050ns	0.038000**	0.005000ns	0.085000ns
TSF×TSF	0.072792ns	0.002321ns	0.039583**	0.050042**	-6.060000**
STPP×MTGase	0.061750ns	0.002600ns	0.001250ns	0.007500ns	-0.555000ns
STPP×TSF	-0.172500*	-0.020075ns	0.000750ns	-0.031750*	-0.350000ns
STPP×STPP	-0.077958 ns	0.023371ns	-0.007167ns	0.004042ns	-4.500000*

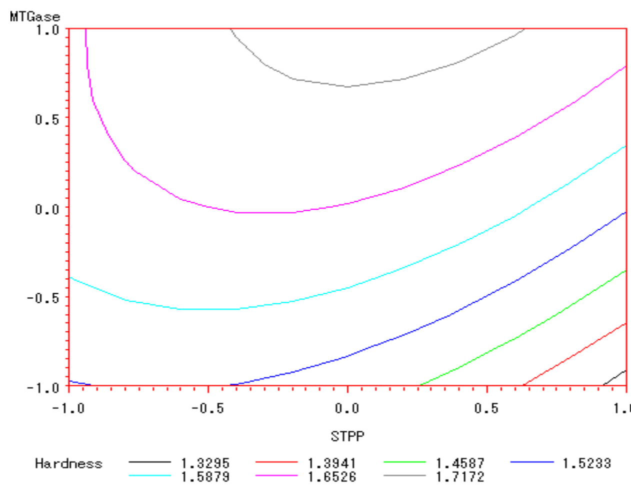
*: Significant at p≤0.05; **: significant at p≤0.01; ***: significant at p≤0.001; ns: not significant

where, X_1 represents the amount of MTGase, X_2 represents the amount of TSF, X_3 represents the amount of STPP and CY represents cooking yield.

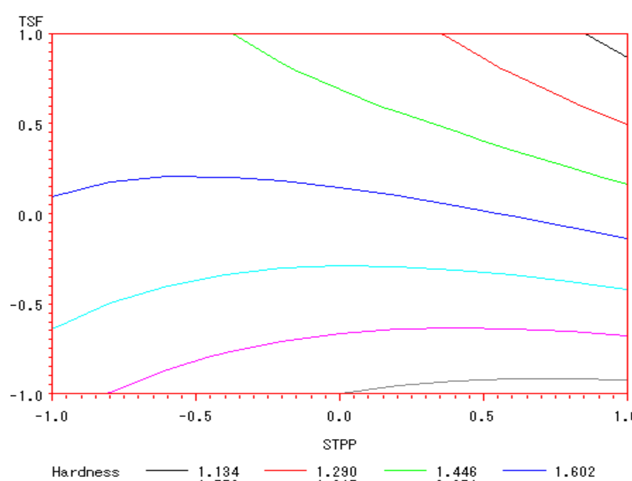
Effects of MTGase, TSF and STPP amounts: The effects of different amounts of MTGase, TSF and STPP on the instrumental data (hardness, springiness, cohesiveness and chewiness) and cooking yield of pork batter gel were reported using the coefficient of the second-order polynomials (Table 3). To aid visualization, we illustrated the contour plots of the response variables in Fig. 1 to 5.

TPA: Instrumental TPA was performed on the cooked pork batter; texture attributes, such as hardness, springiness, cohesiveness and chewiness, were selected as response variables. The hardness and springiness of the cooked pork batter depended on the linear amount effects of the MTGase (positive at $p<0.05$, Fig. 1a; positive at $p<0.01$, Fig. 2a) and TSF (negative at $p<0.001$, Fig. 1b; negative at $p<0.01$, Fig. 2b). This result was due to the added MTGase, resulting in the formation of ε-(γ-glutamyl)lysyl bonds between myofibrillar proteins. The reaction was irreversible and likely contributed to strong protein-protein interactions that stabilize the network (Tseng *et al*., 2000b).

MTGase-induced gelation produces more regular structures than thermal-induced gels (Chanyongvorakul *et al*., 1995). Furthermore, the presence of the enzyme-hydrolyzed soy protein fraction with relatively high content of short peptides influences gel network formation caused by myofibrillar proteins (Feng and Xiong, 2003). This finding may be attributed to the following mechanism. At high ionic strength, myofibrillar protein isolate molecules can be well hydrated and shielded by a double electric layer,

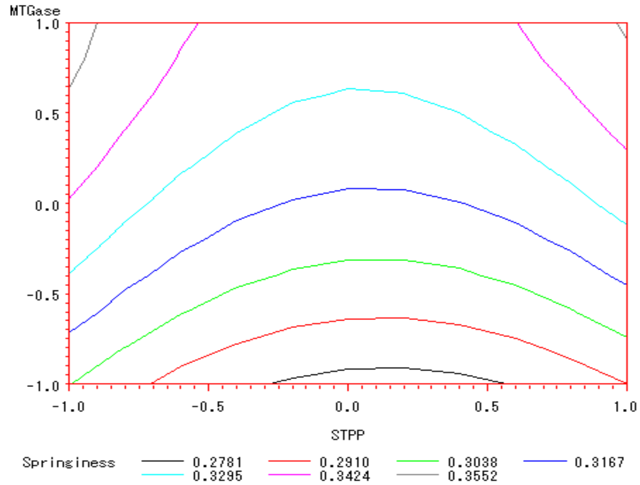


(a)

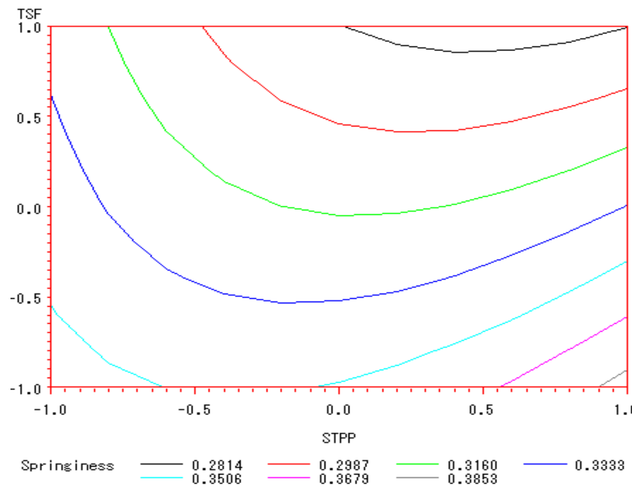


(b)

Fig. 1: Contour plots of the interactive effect of; (a): MTGase and STPP and (b): TSF and STPP on the hardness of cooked pork batter



(a)



(b)

Fig. 2: Contour plots of the interactive effect of; (a): MTGase and STPP and (b): TSF and STPP on the springiness of cooked pork batter

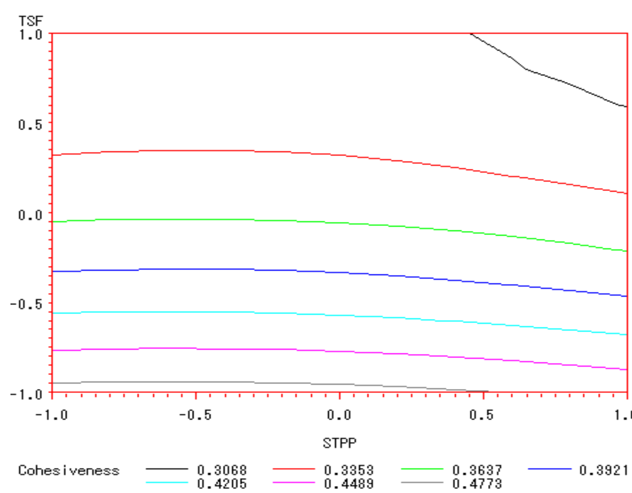
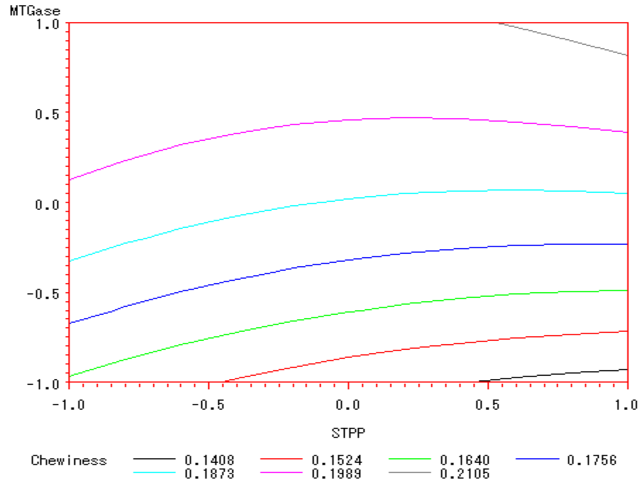
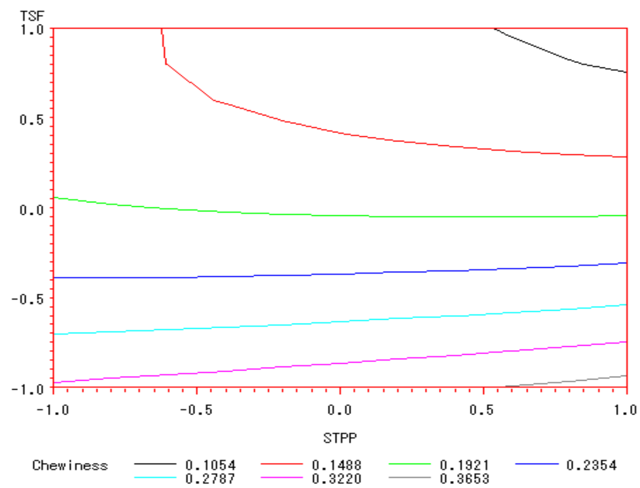


Fig. 3: Contour plots of the interactive effect of TSF and STPP on the cohesiveness of cooked pork batter



(a)



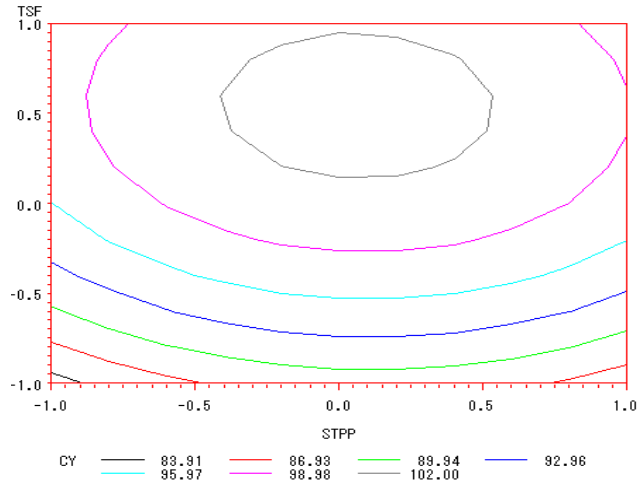
(b)

Fig. 4: Contour plots of the interactive effect of; (a): MTGase and STPP and (b): TSF and STPP on the chewiness of cooked pork batter

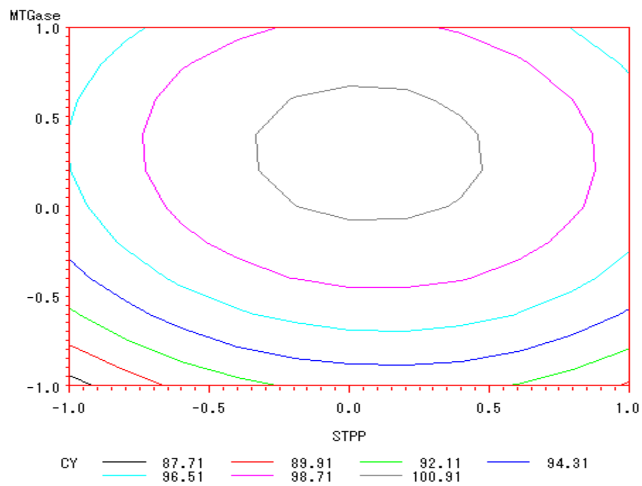
there by reducing MTGase accessibility (Xiong *et al*., 2008). In addition, non-meat proteins can directly interact with meat proteins, occupying the interstitial spaces in a gel matrix or gel as discrete pockets in a matrix structure (Lanier, 1991).

The amount of added STPP did not significantly affect the textural characteristics ($p>0.05$). This effect was altered by the presence of increased levels of enzyme-hydrolyzed soy protein fraction. The interaction between STPP and TSF affected ($p<0.05$) the hardness of the cooked pork batter. The main possible mechanisms associated with phosphate activity in meat were listed as follows: increasing ionic strength; functioning polyanion; shifting pH value from the meat isoelectric range; sequestering metal ions; and dissociating actomyosin to some extent (Sofos, 1986). In hydrolysis, protein molecules are unfolded and cleaved, charged terminals are produced and active amino acid side-chain groups and hydrophobic patches are exposed. These modifications possibly affect the interactions between proteins and interactions between proteins and other food constituents, such as lipids, polysaccharides and flavor compounds. Electrostatic interactions possibly contribute to Denatured State Ensemble (DSE) (Cho *et al*., 2004). However, studies have shown that favorable and unfavorable electrostatic interactions appear in the DSE. These interactions include specific non-native interactions that persist even in the transition state of protein folding. Ionic bonds are significant factors in DSE (Cho *et al*., 2008). Barbut and Mittal (1990) reported that low-salt batters exhibit lower elasticity but higher chewiness and hardness values than high-salt batters.

The amount of TSF showed negative linear ($p<0.001$) and positive quadratic effects ($p<0.01$) on the cohesiveness (Fig. 3) and chewiness (Fig. 4b) of cooked



(a)



(b)

Fig. 5: Contour plots of the interactive effect of; (a): TSF and STPP and (b): MTGase and STPP on the Cooking Yield (CY) of cooked pork batter

pork batter; this result indicates that the enzyme-hydrolyzed soy protein elicits a self-reinforcing effect as the amount of added TSF is increased (Dijkstra *et al*., 2012). The interaction between MTGase and TSF positively affected ($p<0.01$) the cohesiveness of cooked pork batter. The amount of MTGase showed positive linear ($p<0.01$) effects on the chewiness of cooked pork batter (Fig. 4a). Furthermore, the interaction between STPP and TSF weakened ($p<0.05$) the chewiness of cooked pork batter.

Cooking yield: The amount of TSF showed positive linear ($p<0.01$) and negative quadratic effects ($p<0.01$) on the yield of cooked pork batter (Fig. 5a). Soluble myofibrillar proteins extracted using high-ionic strength brines, particularly when these proteins mixed with hydrolyzed soy protein fraction, formed a viscoelastic gel matrix; thus, water was further entrapped in cooked meats. Ionic environment influences the amount of extracted protein, thereby affecting the structural, density and stability of meat products (Gordon and Barbut, 1992). Structural changes were affected by altering the electrostatic and hydrophobic interactions among proteins or by eliciting effects that are dependent on the specific properties of involved ions.

The amount of MTGase also showed positive linear ($p<0.05$) and negative quadratic effects ($p<0.05$) on the yield of cooked pork batter (Fig. 5b). Studies (Kuraishi *et al*., 1998; Motoki and Seguro, 1998) have shown that the formation of ε-(γ-glutamyl)lysyl crosslinks is related to the binding strength of restructured, minced meats or meat gels produced without or with lowered concentrations of NaCl and/or phosphates.

Model fitting from RSM for sensory analysis: Instrumental methods are useful because they are easier to perform, standardize and reproduce; these methods require few trained people. These methods also produce results more quickly than sensory methods. However, instrument calibration should be performed against human senses to obtain accurate results; furthermore, only the human senses can truly perceive, describe and quantify texture (Pereira *et al*., 2005). The results in Table 5 showed that the elasticity model of all of the response variables was highly adequate for the satisfactory levels of R^2 (90.3%); no significant lack-of-fit in all of the response variables (p>0.05) was observed. By contrast, the models of firmness and juiciness were not adequate. Although the satisfactory levels of R^2 were >80%, significant lack-of-fit was observed in response variables (p<0.05).

The model of overall acceptability was also not adequate because it either exhibited unsatisfactory R^2 of >80% (R^2 = 66.7%) or significant lack-of-fit was observed in all of the response variables (p<0.05). Dobraszczyk and Vincent (1999) showed that overall acceptability is a synthetic process to perform sensory analysis, involving the tongue, teeth, cheeks, saliva and food matrix; thus, it may be more complex and important than any other instrumental or sensory partial parameter.

Correlation coefficients: Table 6 shows that the Pearson correlation coefficients of hardness and springiness, cohesiveness, chewiness, firmness and elasticity were 0.74 (p<0.01), 0.80 (p<0.001), 0.94 (p<0.001), 0.90 (p<0.001) and 0.72 (p<0.01), respectively. The Pearson correlation coefficients of springiness and hardness, cohesiveness, chewiness, firmness and elasticity were 0.74 (p<0.01), 0.59 (p<0.05), 0.81 (p<0.001), 0.75 (p<0.01) and 0.86 (p<0.001), respectively. Yuan and Chang (2007) reported that hardness values obtained by both levels of penetration correlate significantly (R^2 = 0.94 to 0.95) with sensory hardness. Springiness obtained using the 50% penetration method did not correlate significantly with the sensory data, whereas 75% penetration significantly correlated with the sensory data (R^2 = 0.98). Researchers (Lee and Resurreccion, 2002) demonstrated that the degree of penetration and crosshead speed may be a significant factor affecting the Pearson correlation coefficients. In the present study, the sample was placed under the probe that moved downward at a constant speed of 3.0 mms^{-1} (pre-test), 1.0 mms^{-1} (test), 3.0 mms^{-1} (post-test) and 75% penetration.

Lassoued *et al*. (2008) revealed that the texture of crumb as felt feels by touch or in the mouth is greatly influenced by the size or the structure of the crumb cells; for instance, finer, thin-walled, uniformly sized cells yield a softer and more elastic texture than coarse, open and thick-walled cell structures, which are affected by cohesiveness. The Pearson correlation coefficients of cohesiveness and hardness, springiness, chewiness, cooking yield, firmness, elasticity and juiciness were 0.80 (p<0.001), 0.59 (p<0.05), 0.90 (p<0.001), -0.70 (p<0.01), 0.69 (p<0.01), 0.65 (p<0.01), -0.68 (p<0.01), respectively. To our

Table 5: ANOVA showing the linear and quadratic interactions and the lack-of-fit of the response variables for sensory evaluation

		Response variables							
		Firmness (panel)		Elasticity (panel)		Juiciness (panel)		Overall acceptability (panel)	
Sourse of variation	Df	Sequential sum of squares	*F*	Sequential sum Of squares	*F*	Sequential sum Of squares	*F*	Sequential sum of squares	*F*
Regression									
Linear	3	2.078025	88.60^{***}	0.212050	10.24^{*}	0.079425	17.67^{**}	5.1475	1.17^{ns}
Quadratic	3	0.096892	4.13^{ns}	0.042135	2.04^{ns}	0.027218	6.06^{*}	8.524333	1.93^{ns}
Cross product	3	0.636125	27.12^{**}	0.068275	3.3^{ns}	0.000625	0.14^{ns}	1.085	0.25^{ns}
Total model	9	2.811042	39.95^{***}	0.322460	5.19^{*}	0.107268	7.95^{*}	14.756833	1.11^{ns}
Lack-of-fit	3	0.038625	55.18^{*}	0.03130	6.52^{ns}	0.007425	74.25^{*}	7.3225	154.16^{**}
R^2 (%)		98.6		90.3		93.5		66.700	

*: Significant at p≤ 0.05; **: significant at p≤ 0.01; ***: significant at p≤ 0.001; ^{ns}: not significant; Df = Degree of Freedom; *F* = rate of variance estimates

Table 6: Pearson correlation coefficients of hardness, springiness, cohesiveness, chewiness, cooking yield, firmness, elasticity, juiciness and overall acceptability

	Hardness	Springiness	Cohesiveness	Chewiness	Cooking yield	Firmness	Elasticity	Juiciness	Overall acceptability
Hardness	1.00								
Springiness	0.74^{**}	1.00							
Cohesiveness	0.80^{***}	0.59^{*}	1.00						
Chewiness	0.94^{***}	0.81^{***}	0.90^{***}	1.00					
Cooking yield	-0.45	-0.38	-0.70^{**}	-0.64^{*}	1.00				
Firmness	0.90^{***}	0.75^{**}	0.69^{**}	0.81^{***}	-0.24	1.00			
Elasticity	0.72^{**}	0.86^{***}	0.65^{**}	0.75^{**}	-0.26	0.81^{***}	1.00		
Juiciness	-0.44	-0.26	-0.68^{**}	-0.60^{*}	0.96^{***}	-0.21	-0.23	1.00	
Overall acceptability	0.06	0.04	-0.15	-0.04	0.38	0.03	0.15	0.38	1.00

Coefficients from three replications (*n* = 15); *: Significant at p≤ 0.05; **: significant at p≤ 0.01; ***: significant at p≤ 0.001

knowledge, texture is a group of physical properties derived from the structure of food; the mechanism by which the constituent ingredients of food interact is related to cohesiveness, which can be associated with the perception of texture by consumers (Farouk *et al*., 2002; Pereiraa *et al*., 2003).

Chewiness denotes the energy required to chew a solid sample to a steady state of swallowing (Chéret *et al*., 2005). The results showed that the Pearson correlation coefficients of chewiness and hardness, springiness, cohesiveness, cooking yield, firmness, elasticity and juiciness were 0.94 ($p<0.001$), 0.81 ($p<0.001$), 0.90 ($p<0.001$), -0.64 ($p<0.05$), 0.81 ($p<0.001$), 0.75 ($p<0.01$) and -0.60 ($p<0.05$), respectively. The Pearson correlation coefficients of cooking yield and cohesiveness, chewiness and juiciness were -0.70 ($p<0.01$), -0.64 ($p<0.05$) and 0.96 ($p<0.001$), respectively. Furthermore, the Pearson correlation coefficient of firmness and elasticity was 0.81 ($p<0.001$). Researchers indicated that sensory hardness is significantly correlated with instrumental hardness, cohesiveness, gumminess and chewiness. Positive linear correlations between instrumental and sensory hardness are found in milk chocolate (Nightingale *et al*., 2009), bread (Gambaro *et al*., 2002), cheese (Hough *et al*., 1996) and tomatoes (Lee *et al*., 1999).

Overall acceptability was poorly correlated with instrumental attributes and partial attribute; this result is consistent with that in previous studies. Lee *et al.* (1999) and Lee and Resurreccion (2002) reported that instrumental parameters, such as hardness, cohesiveness and gumminess, are not related to the residual cohesiveness of mass. The lack of relationship may be attributed to the process of complete mastication, which is not imitated by the instrument (Everard *et al*., 2006; Kim *et al*., 2009). Ta´rrega and Costell (2007) indicated that the samples showing intermediate values of both sensory attributes are preferred; thus, the overall acceptability is not satisfactorily predicted by any of the instrumental parameters.

CONCLUSION

The stimulatory effects of enzyme-hydrolyzed soy proteins and MTGase on the cooking yield of cooked pork batter were more evident than STPP. This result suggested that enzyme-hydrolyzed soy protein and MTGase could be used as binders in products. With binders, products can be easily cut and reproduce the sensations contributed by fat, if low salt is desired by consumers. Furthermore, the cooking yield of cooked pork batter was mainly affected by instrumental cohesiveness and chewiness. In short, enzyme-hydrolyzed soy protein fraction (molecular weight cut-off = 0.5 kDa to 10 kDa) could improve the overall acceptability of cooked pork batter added with MTGase to optimize satisfactory sensory attributes and healthy food.

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