HYBRID CATFISH GEL FORMATION AND ITS TEXTURAL PROPERTIES

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ABSTRACT

Response surface methodology (RSM) was employed to study the effect of pre-incubation temperature (25 to 65 ^{0}C) and time (60 to 240 minutes) on the biochemical and textural properties of gels prepared from hybrid catfish (Clarias gariepinus x Clarias macrocephalus). Pre-incubation temperature affected gel solubility, trichloroacetic soluble peptides (TCA-soluble peptides) and textural properties in quadratic, linear and quadratic manner ($P<0.05$), respectively. There was a significant effect ($P<0.05$) of pre-incubation temperature on changes in biochemical and textural properties of gels. Each predictive model showed a significant lack of fit ($P<0.05$), indicating that the models were not adequate for prediction.

The estimation response surfaces for gel solubility showed that an increase in pre-incubation temperature decreased gels solubility. The lowest solubility of pre-incubated sample and cooked gel were found at pre-incubation temperature ranging from 35 to 50 ^{0}C for 100 to 220 minutes. At pre-incubation temperature above 50 ^{0}C, an increasing in solubility of pre-incubation sample and cooked gel was observed. An increasing of pre-incubation temperature influenced on an increase in TCA-soluble peptides value of pre-incubated sample and cooked gel. Breaking force and deformation of cooked gels increased as pre-incubation temperature increased. At setting temperature above 50 ^{0}C, a decrease in breaking force and deformation of cooked gels were observed. The optimum condition for pre-incubation of hybrid catfish minced gel was found to be 45 ^{0}C for 150 minutes before pre-incubated sample was cooked at 90 ^{0}C for 30 minutes.

Keywords: modori; fish protein gelation; textural properties; hybrid catfish

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INTRODUCTION

The most important characteristic of minced fish or surimi is its ability to form highly textural properties of gels when mixed with salt and other ingredients (Babbitt and Reppond 1988). Gel setting has been applied in the surimi industry for a long time to increase the textural qualities of surimi gels (Benjakul, Chantarasuwan, and Visessanguan 2003). Setting of fish paste with or without subsequent heating resulted in increases in both breaking force and deformation of pre-incubation sample and cooked gels (Benjakul and Visessanguan 2003). Heat-induced gelation procedure has been recognized as one of the critical steps that can be controlled to improve the gel quality of fish flesh (Luo, Xiong, Wang, and Mims 2000). Several researchers studied on thermal gelation of fish mince and myofibrillar proteins, especially concerning thermal behavior of surimi gel. Temperature plays an important role in fish protein gelation. In addition to its effects on the conformation of myofibrillar proteins, temperature can activate endogenous enzymes that naturally occur in fish muscle (An, Peters, and Seymour 1996; Totosaus, Montejano, Salazar, and Guerrero 2002). Shimizu, Machida, and Takenami (1981) reported that fish muscles from various species showed similar reactions to temperature. Two important phenomena occurring during heat-induced gelation consist of setting and thermal-associated gel degradation (modori) (Uresti, Ramírez, Lopez-Arias, and Vazquez 2003). Ho, Chen, and Jiang (2000) suggested that refrigerated (0 - 5 ^{0}C), ambient (25 - 30 ^{0}C) and warm temperatures (50 – 65 ^{0}C) are usually used for setting of washed mince gels. Heat-induced gelation at different temperatures may lead to different gel characteristics, especially with different fish species (Benjakul, Visessanguan, Tueksuban, and Tanaka 2003). Fish paste is incubated at temperature lower than 40 ^{0}C involve polymerization of myosin at low temperature due to the formation of covalent non-disulfide cross-linking induced by endogenous transglutaminase (TGase) (Joseph, Lanier, and Hamann 1994; An and others 1996; Yongsawatdigul, Worratao, and Park 2002; Benjakul and others 2003; Uresti and others 2003; Benjakul and Visessanguan 2003; Benjakul, Visessanguan, and Chantarasuwan 2004; Uresti, Velazquez, Vazquez, Ramírez, and Torres 2006). TGase has been known to catalyze an acyl-transfer reaction in which the γ-carboxyamide groups of peptide-bound glutaminyl residues are the acyl donors. A variety of primary amines and the lysyl residues of proteins could act as acyl acceptors, the later generating ε-(γ-glutamyl) lysine cross-links (Lee, Lanier, Hamann, and Knopp 1997). Modori is a term used to describe thermal-associated gel degradation when fish paste is incubated at temperatures close to 60 ^{0}C (Alvares, Couso, and Tejada 1999; Ramires, Garcia-Carreno, Morales, and Sanchez 2002; Uresti and others 2003). It has been suggested that proteolytic degradation of myofibrillar proteins, especially myosin heavy chain affected gel-forming properties of minced fish (GÓmez-Gullén, Martínez-Alavarez, and Montero 2003). The breakdown of myofibrillar proteins inhibits the development of three-dimensional gel network. In general, weakening of surimi gels occurs at temperature above 50 - 70 ^{0}C (Benjakul, Visessanguan, and Tueksuban 2003). Endogenous serine and cysteine muscle proteinases have been associated with the degradation of gel structure at a high temperature (Uresti and others 2003). Heat-induced gelation response of fish gel can be varied, depending on fish species. The optimum heating tempera-

ture among species may be determined by the heat stability of myosin and characteristics of TGase (Benjakul and others 2003). Many researchers have reported the effects of heating temperature and heating period on protein gel forming of washed mince or surimi gels (Luo, Kuwahara, Kaneniwa, Murata, and Yokoyama 2001). There are a few researchers reported the effect of heating conditions on minced fish gel especially, freshwater fish. Understanding the effect of temperature and time on chemical and textural properties changes of minced hybrid catfish gel would lead to an appropriate guideline of heat-induced gelation process and better quality control for fishery products development from hybrid catfish.

The objective of this study was to investigate the effect of temperature and time during heat-induced gelation process on changes of chemical and textural properties of hybrid catfish minced gel using response surface methodology.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid (TCA) was purchased from Carlo Erba Reactifs (Rodano, Italy). Sodium dodecyl sulfate (SDS) was purchased from Ajex Finechem (NSW, Australia). Other chemicals were analytical grade and purchased from Fluka Company (Buchs, Switzerland) and Carlo Erba Reactif (Rodano, Italy).

Samples preparation

Hybrid catfish (Clarias gariepinus x Clarias macrocephalus) were obtained from an aquatic farm in Kalasin province, Thailand. Six-month old fish with weight about 150 - 175 g each were transported to a fish processing plant (Kalasin sausage Co., Ltd.) within 20 minutes and kept alive for 12 hours before being processed. Fish were filleted, deskinned and eviscerated by experienced workers. The fillets were packed in polyethylene bag. The crushed ice was added at the bottom of plastic box. The sample bag was placed in plastic box and then the crushed ice was added on the top of the plastic box to cover the sample, and immediately transported to the Khon Kaen University Food Processing Laboratory within 2 hours. The fillets were repacked 1 kg each in polyethylene bag, and stored at -30 ^{0}C until use.

Minced fish gel preparation

Fish fillet was chilled at 0 ^{0}C for 2 hours. Fish flesh (500 g) was minced using a blender (Matsushita Electric Industrial Co., Ltd., Selangor Darul Ehsan, Malaysia) for 15 minutes with 5 stops for 0.5 minutes to scrape the bowl. Sodium chloride and ice were added to obtain a sol with 2 % salt and 80 % moisture content. During chopping, the temperature of sol was maintained below 15 ^{0}C. The sol was vacuum-packed (Supervac; Busch, Germany) in a polyethylene bag and consequently stuffed into 2.3

cm diameter stainless steel tube using a stuffer (Dick; D73728, Esslingen, Germany). The sample was pre-incubated at temperature and time in water bath (Thermo Haake, Karlsruhe, Germany) as shown in Table 1. The sample of each treatment was separated into two groups. First group (pre-incubated sample) was immediately chilled in iced water for 20 minutes before gel solubility and trichloroacetic acid soluble peptides analysis. Second group (cooked gel) was immediately submerged at 90 ^{0}C for 30 minutes, then chilled in iced water for 20 minutes and kept at 4 ^{0}C overnight before solubility, trichloroacetic acid soluble peptides and texture measurement.

Table 1 : Codified and decodified variables established according to the central composite design for setting conditions of minced gel.

Treatment	Codified		Decodified	
	Time	Temperature	Time (min)	Temperature (^{0}C)
1	0	0	150.0	45.0
2	-1.414	0	22.7	45.0
3	1	1	240.0	65.0
4	0	0	150.0	45.0
5	0	0	150.0	45.0
6	1	-1	240.0	25.0
7	-1	-1	60.0	25.0
8	1.414	0	277.3	45.0
9	0	1.414	150.0	73.3
10	0	-1.414	150.0	16.7
11	0	0	150.0	45.0
12	0	0	150.0	45.0
13	-1	1	60.0	65.0

Determination of solubility

Solubility of gel was determined by method described by Benjakul, Visessanguan, and Chantarasuwan (2004). Pre-incubated or cooked gel sample (1 g) was homogenized in 20 ml of 20 mM Tris-HCl, pH 8.0, containing 1 % (w/v) SDS, 8 M urea and 2 % (v/v) β-ME for 1 minute using a laboratory blender (Waring Commercial, Connecticut, USA). The homogenate was heated in boiling water (100 ^{0}C) for 2 minutes and stirred at room temperature for 4 hours. The resulting homogenate was centrifuged at 10,000 x g for 30 minutes (Beckman Instruments, Inc., California, USA). Protein in supernatant (10 ml) was precipitated by the addition of 50 % (w/v) cold TCA to obtain a final concentration of 10 %. The mixture was kept at 4 ^{0}C for 18 hours and then centrifuged at 10,000 x g for 30 minutes. The precipitate

was washed with 10 % TCA and solubilized in 0.5 M NaOH. To obtain the total amount of protein, gel was directly solubilized in 0.5 M NaOH. The protein content was measured using Lowry method (Lowry, Rosebrough, Fan, and Randall 1951). The solubility was expressed as percent of the total protein.

Determination of trichloroacetic acid soluble peptides (TCA-soluble peptides)

TCA-soluble peptides were determined according to the method described by Benjakul and others (2003). Pre-incubated or cooked gel (3 g) was homogenized with 27 ml of 5 % TCA (w/v). The homogenate was kept in ice for 1 hour and centrifuged at 5,000 x g for 5 minutes. The soluble peptides in the supernatant were measured by Lowry method (Lowry and others 1951) and expressed as μmol tyrosine/ g sample.

Texture analysis

Puncture test was carried out using a Texture Analyzer (TA.XT Plus, Stable Micro System, Surrey,UK) with 5 mm spherical probe (SMS P/5S) using 25 kg load cell, at a test speed of 1.0 mm/s. Five samples were cut into 2.3 cm long. Puncture force (g) and deformation (mm) were recorded.

Experimental design

Response surface methodology (RSM) was employed to study the effect of pre-incubation conditions on biochemical and textural properties changes of hybrid catfish minced gel. Hybrid catfish minced gels were prepared according to a central composite design (CCD), consisting of 22 factorial design with two levels (-1, +1), five central points (0) and two levels of axial points ($-\alpha$, $+\alpha$). The experimental design adopted 2 independent variables being the pre-incubation temperature (T) and pre-incubation time (t) of pre-incubation conditions. The treatments in this design were set using a statistical program (Design-expert Version 5.0.8), resulting in 13 treatments as shown in Table 1. The dependent variables (responses) were: the solubility and TCA-soluble peptides of pre-incubated samples (pre-incubation without cooking at 90 ^{0}C for 30 minutes) and cooked gels (pre-incubation with cooking at 90 ^{0}C for 30 minutes), as well as breaking force and deformation of cooked gels. All data presented were mean values of three determinations. Three-dimensional response surface plots were generated using a statistical program (Statistica Softwere Version 6.0).

RESULTS AND DISCUSSION

To find the effect of pre-incubation temperature and pre-incubation time of minced gels, all properties of pre-incubated samples and cooked gels obtained by response surface methodology over a range of pre-incubation temperature (25 - 65 ^{0}C) and pre-incubation time (60 - 240 minutes) were collected. The correlations of pre-incubation conditions on solubility of pre-incubated samples and cooked gels were found as quadratic terms ($P<0.05$) with coefficient of determination (R2) of 0.8166 and 0.8395, respectively (Table 2). TCA-soluble peptides of pre-incubation samples and cooked gels

were found as linear terms ($P<0.05$) with R2 of 0.6473 and 0.6760, respectively (Table 3). In the case of breaking force and deformation of cooked gels, the correlation of pre-incubation temperature and time was found as quadratic terms ($P<0.05$) with R2 of 0.7224 and 0.7034, respectively (Table 4).

As can be found in Table 5, 6 and 7, the pre-incubation temperature significantly influenced ($P<0.05$) the solubility (quadratic term) and TCA-soluble peptides (linear term) of pre-incubated samples and cooked gels as well as breaking force and deformation (quadratic terms) of cooked gels, indicating that changes in the limits of temperature studied in this experiment, contributed to the change in all response parameters. On the other hand, the pre-incubation time did not significantly ($P>0.05$) influence all response parameters. Mathematical models expressing the correlation of pre-incubation temperature on solubility, TCA-soluble peptides of pre-incubated samples and cooked gels as well as breaking force and deformation of cooked gels were shown in equation 1 to 6.

Solubility of pre-incubation sample = 90.94 + 0.78 (T) + 4.18 (T)2 (R2 = 0.8166) (1)

Solubility of cooked gel = 89.49 + 0.73 (T) + 4.54 (T) 2 (R2 = 0.8395) (2)

TCA-soluble peptides of pre-incubation sample = 0.25 + 0.049 (T) (R2 = 0.6473) (3)

TCA-soluble peptides of cooked gel = 0.26 + 0.050 (T) (R2 = 0.6760) (4)

Breaking force of cooked gel = 316.58 – 27.25 (T) – 50.31 (T) 2 (R2 = 0.7224) (5)

Deformation of cooked gel = 10.95 - 0.61 (T) - 1.36 (T) 2 (R2 = 0.7034) (6)

T = Pre-incubation temperature

Equations 1 to 6 revealed that changes in pre-incubation temperature took into account of changes in solubility and TCA-soluble peptides of pre-incubated samples and cooked gels as well as breaking force and deformation of cooked gels. Each predictive model showed a significant lack of fit ($P<0.05$), indicating that the models were not adequate for prediction (Table 2-4).

The solutions containing β-mercaptoethanol, urea and sodium dodecyl sulfate (SDS) were used to solubilize protein by destroying all bonds, except non-disulfide covalent bonds, particularly the $\varepsilon(\gamma$-glutamyl) lysine linkage (Benjakul, Visessanguan, and Srivilai 2001; Benjakul and Visessanguan 2003; Benjakul and others 2003). It is known that, during the pre-incubation phenomenon, fish protein aggregates, inducing the gel formation (Niwa, Ueno, and Kanoh 1992; Morales, Ramirez, Vivanco, and Vazquez 2001). The decrease in solubility was due to the formation of non-disulfide covalent cross-linking presumably induced by endogenous transglutaminase (TGase) during pre-incubation. The differences of solubility were possibly caused by differences of TGase activity at pre-incubation condition.

The estimated response surfaces were plotted for solubility of pre-incubated samples and cooked gels of the hybrid catfish (Figure 1 and 2). The results showed that an increase in pre-incubation temperature resulted in decreases in solubility of pre-incubated samples and cooked gels. The lowest solubilities of pre-incubated samples and cooked gels were found over the pre-incubation temperature ranging from 35 to 50 ^{0}C for 100 to 220 minutes. According to the result of endogenous TGase temperature profile (data not shown), the highest activity of TGase was found at 60 ^{0}C. However, the activity of TGase was still high at temperature ranging from 30 to 50 ^{0}C. At pre-incubation temperature above 50 ^{0}C, an increase in solubility of pre-incubated samples and cooked gels were observed. This possibly due to the activity of TGase was inactivated. In addition, the pre-incubation temperature rose nearly the highest activity of endogenous proteinases of hybrid catfish at 65 ^{0}C. Thus, an increase in solubility of pre-incubated sample and cooked gel was found when the sample was pre-incubation at above 50 ^{0}C. Cooked gel solubility was slightly lower than that of pre-incubated sample. This result was in reasonable agreement with Benjakul and others (2003) who reported that at the same pre-incubation condition, cooked gels from bigeye snapper, bigeye croaker, threadfin bream and barracuda washed mince showed lower solubilities than pre-incubation samples from the same species. Since, during heating up to 90 ^{0}C, some non-disulfide covalent cross-links were formed, until TGase was inactivated by increasing temperature, resulting in a slightly lower solubility (Benjakul and others 2004). At higher temperatures, proteins underwent unfolding, allowing the reactive lysine or glutamine residues exposed for $\varepsilon(\gamma$-glutamyl) lysine linkage formation. In addition, proteinases were inactivated at higher temperature and caused no further degradation (Benjakul and others 2004). These phenomena were coincidental with an increase and a decrease in textural properties of gels, respectively.

Table 2 : Analysis of variance of the effect of the pre-incubation condition variables on solubility of pre-incubated samples and cooked gels

Source	Sum of squares	DF	Mean square	F value	Prob>F
Solubility of pre-incubated sample					
Linear	11.11	2	5.56	0.36	0.7037
Quadratic	122.64	3	40.88	9.52	0.0072
Residual	23.06	5	4.61	-	-
Lack of fit test for quadratic	26.65	3	8.88	10.45	0.0231
R^2	0.8166				

Table 2 : Analysis of variance of the effect of the pre-incubation condition variables on solubility of pre-incubated samples and cooked gels (continue)

Source	Sum of squares	DF	Mean square	F value	Prob>F
Solubility of cooked gel					
Linear	8.62	2	4.31	0.25	0.7870
Quadratic	146.08	3	48.69	11.53	0.0042
Residual	21.68	5	4.34	-	-
Lack of fit test for quadratic	28.92	3	9.64	59.08	0.0009
R^2	0.8395				

Table 3 : Table 3 Analysis of variance of the effect of the pre-incubation condition variables on TCA-soluble peptides of pre-incubated samples and cooked gels

Source	Sum of squares	DF	Mean square	F value	Prob>F
TCA-soluble peptides of pre-incubated sample					
Linear	0.022	2	0.011	9.18	0.0055
Quadratic	1.092E-03	3	3.642E-04	0.23	0.8722
Residual	6.908E-03	5	1.382E-03	-	-
Lack of fit test for linear	0.012	6	2.023E-03	5310.10	<0.0001
R^2	0.6473				
TCA-soluble peptides of cooked gel					
Linear	0.024	2	0.012	10.43	0.0036
Quadratic	8.388E-04	3	2.796E-04	0.19	0.9024
Residual	6.166E-03	5	1.233E-03	-	-
Lack of fit test for linear	0.011	6	1.889E-03	1259.60	<0.0001
R^2	0.6760				

Table 4 : Analysis of variance of the effect of the pre-incubation condition variables on breaking force and deformation of cooked gels

Source	Sum of squares	DF	Mean square	F value	Prob>F
Breaking force of cooked gel					
Linear	6007.88	2	3003.94	0.86	0.4509
Quadratic	23469.78	3	7823.26	4.84	0.0396
Residual	4317.31	5	863.46	-	-
Lack of fit test for quadratic	11308.86	3	3769.62	940.76	<0.0001
R^2	0.7224				
Deformation of cooked gel					
Linear	2.97	2	1.48	0.59	0.5717
Quadratic	16.77	3	5.59	4.70	0.0421
Residual	4.94	5	0.99	-	-
Lack of fit test for quadratic	8.31	3	2.77	1601.75	<0.0001
R^2	0.7034				

Table 5 : Regression coefficient of the pre-incubation condition variables on solubility of pre-incubated samples and cooked gels

Factor	Coefficient Estimate	DF	Standard Error	Prob>ltl
Solubility of pre-incubated sample				
Intercept	90.94	1	0.93	-
A-Temperature	0.78	1	0.73	0.3213
B-Time	-0.88	1	0.73	0.2676
A2	4.18	1	0.79	0.0011
B2	0.77	1	0.79	0.3612
AB	0.47	1	1.04	0.6605
Solubility of cooked gel				
Intercept	89.49	1	0.92	-
A-Temperature	0.73	1	0.73	0.3485
B-Time	-0.74	1	0.73	0.3437
A2	4.54	1	0.78	0.0007
B2	1.08	1	0.78	0.2073
AB	0.57	1	1.03	0.5964

Table 6 : Regression coefficient of the pre-incubation condition variables on TCA-soluble peptides of pre-incubated samples and cooked gels

Factor	Coefficient Estimate	DF	Standard Error	Prob>ltl
TCA-soluble peptides of pre-incubated sample				
Intercept	0.25	1	9.664E-03	-
A-Temperature	0.049	1	0.012	0.0025
B-Time	0.019	1	0.012	0.1552
TCA-soluble peptides of cooked gel				
Intercept	0.26	1	9.341E-03	-
A-Temperature	0.050	1	0.012	0.0017
B-Time	0.020	1	0.012	0.1201

Table 7 : Regression coefficient of the pre-incubation condition variables on breaking force and deformation of cooked gels

Factor	Coefficient Estimate	DF	Standard Error	Prob>ltl
Breaking force of cooked gel Intercept				
A-Temperature	316.58	1	17.99	-
B-Time	-27.25	1	14.22	0.0969
A2	2.95	1	14.22	0.8417
B2	-50.31	1	15.25	0.0131
AB	-27.21	1	15.25	0.1176
	-26.67	1	20.11	0.2264
Deformation of cooked gel				
Intercept	10.95	1	0.49	-
A-Temperature	-0.61	1	0.39	0.1581
B-Time	0.011	1	0.39	0.9787
A2	-1.36	1	0.41	0.0135
B2	-0.81	1	0.41	0.0923
AB	-0.54	1	0.55	0.3507

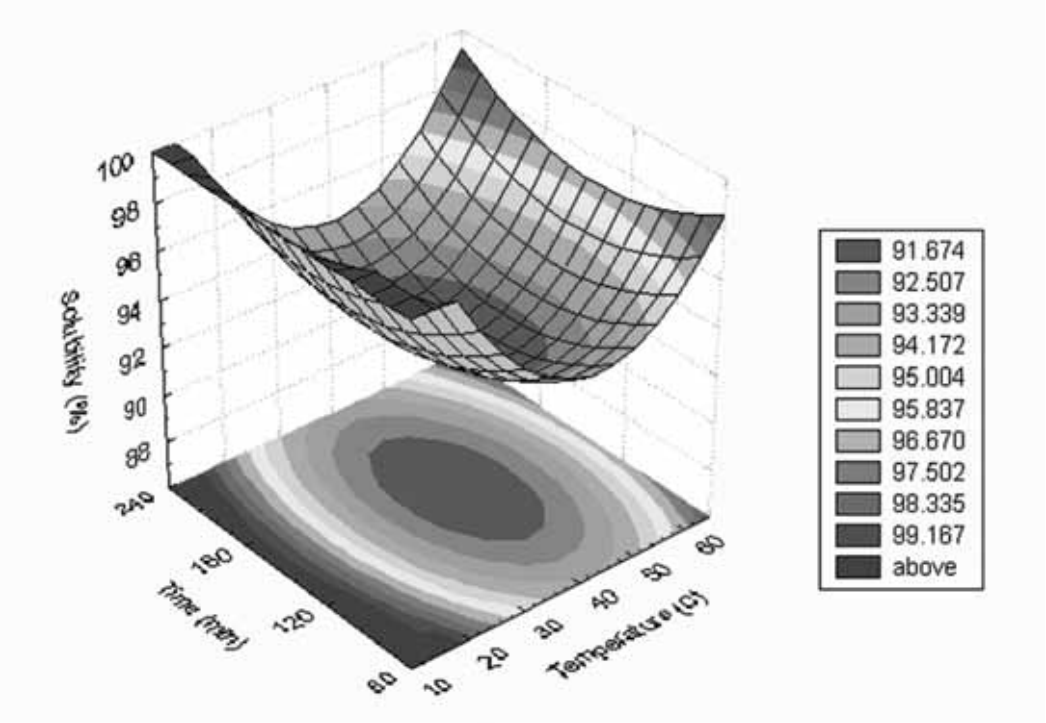

Figure 1 : Response surface plot for solubility of pre-incubated samples with different pre-incubated temperature and time

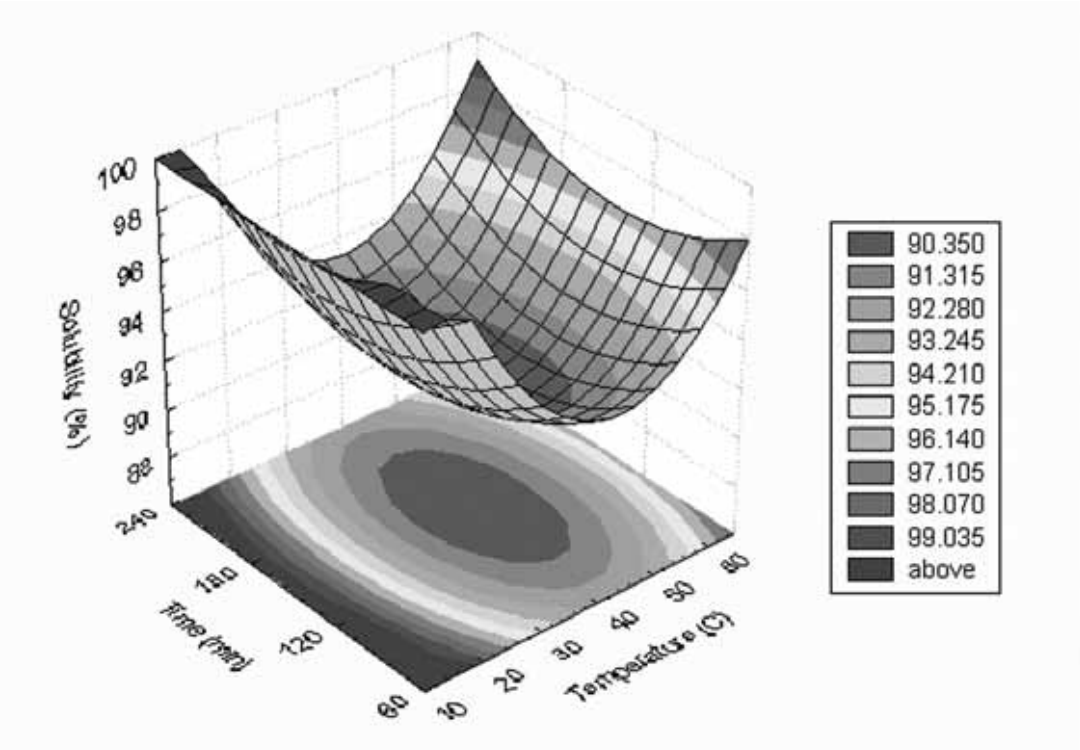

Figure 2 : Response surface plot for solubility of cooked gels with different pre-incubated temperature and time

The TCA-soluble peptides content indicates proteolytic degradation occurred during pre-incubation (Benjakul and others 2004). The breakdown of myofibrillar proteins inhibits the development of three-dimentional gel network (Morrissey, Wu, Lin, and An 1993). Proteolytic activity in fish muscle is still high at 50 - 60 ^{0}C and causes rapid and severe degradation of myofibrillar proteins, particularly myosin called “modori” (Morrissey and others 1993; An and others 1996; Benjakul and others 2004). Figure 3 and 4 showed the estimated response surfaces of pre-incubation samples and cooked gels for TCA-soluble peptides. An increase of pre-incubation temperature influenced an increase in TCA-soluble peptides value of pre-incubation samples and cooked gels. TCA-soluble peptides of cooked gel were higher than pre-incubation sample. There was no significant effect ($P>0.05$) of pre-incubation time on TCA-soluble peptides of pre-incubated sample and cooked gel. However, TCA-soluble peptides value of both slightly increased when pre-incubation time was lengthened. Gel softening is generally caused by two major groups of proteinases, cysteine proteinase (mainly cathepsin) and heat-stable alkaline proteinases at the temperature around 50 - 70 ^{0}C (An and others 1996; Visessanguan, Menino, Kim, and An 2001; Benjakul and others 2003). Jiang (2000) suggested that this phenomenon is induced by endogenous thermal stable proteinases. Benjakul and others (2003) reported that the highest degradation of myosin heavy chain from lizardfish during heating process was observed at 60 ^{0}C, presumably caused by the heat-activated proteinases. Proteolytic activity of minced lizardfish increased with temperature and reached the maximum at 65 ^{0}C (Yongsawatdigul and Piyadhammaviboon 2004). The degree of degradation increased as the incubation time and/or temperature increased. The increase in TCA-soluble peptides coincided with the increase in solubility, especially when the pre-incubation time increased (Benjakul and others 2004). Our previous study showed that the optimum proteinases activities in hybrid catfish muscle were observed at temperature and pH of 65 ^{0}C and 9, respectively. Therefore, proteinases in hybrid catfish muscle possibly were heat-stable alkaline proteinase. Degree of inhibition of proteinase was highest (74.85 %) in the presence of phenylmethylsulfonyl fluoride (PMSF), indicating that serine proteinase(s) was a major group of proteinases. In this study, the highest TCA-soluble peptides of pre-incubated samples and cooked gels were found at the pre-incubation temperature of 65 ^{0}C. This could possibly caused by the activities of serine proteinases.

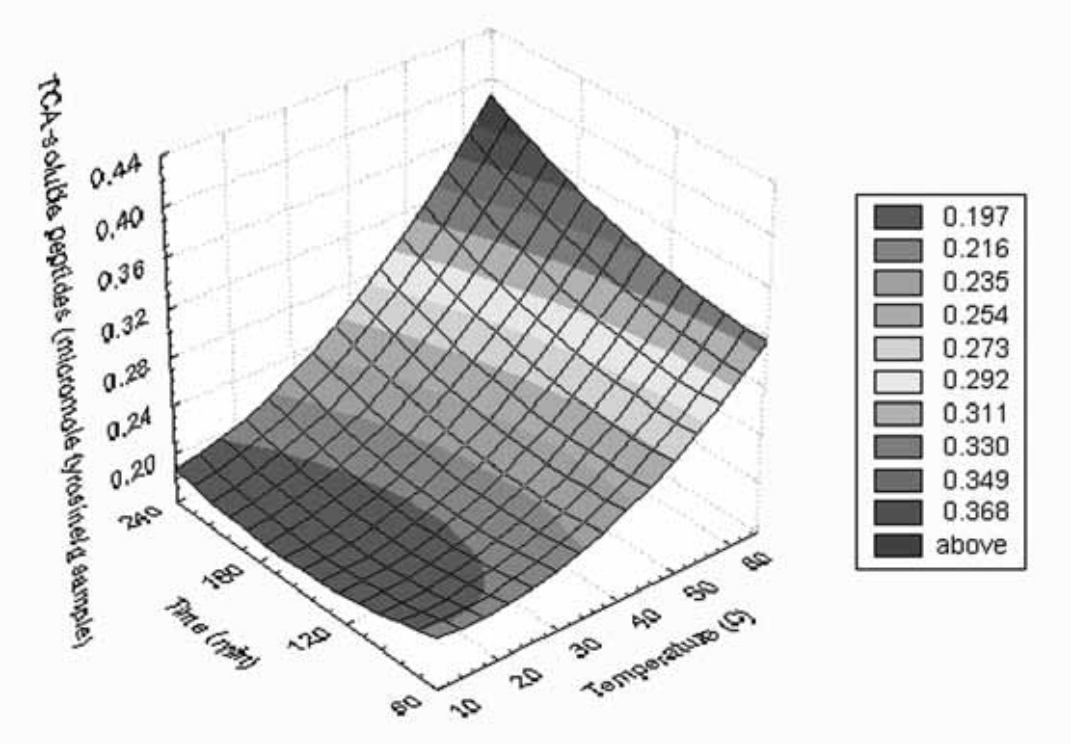

Figure 3 : Response surface plot for TCA-soluble peptides of pre-incubated samples with different pre-incubated temperature and time

Figure 5 and 6 showed the estimated responses surfaces and contour maps of breaking force and deformation of cooked gels. It was found that breaking force and deformation of cooked gels increased along with pre-incubation temperature increased. The highest breaking force and deformation were found at pre-incubation temperature around 27 to 50 ^{0}C for 65 to 230 minutes. At pre-incubation temperature above 50 ^{0}C, breaking force and deformation of cooked gels decreased. A lowest breaking force of cooked gel was found at 65 ^{0}C corresponding to an increase in TCA-soluble peptides content (Figure 3 and 4). There was no significant effect ($P>0.05$) of pre-incubation time on breaking force and deformation of gels. The results indicated that the pre-incubation time might not be the main factor influencing the textural properties of minced gel from hybrid catfish. However, from the estimated response surfaces the breaking force and deformation of cooked gels slightly increased when the pre-incubation time increased. Ramires, Rodriguez-Sosa, Morales, and Vazquez (2003) reported that no influence was found for second order term of time on gelling properties during pre-incubation at temperature ranging from 25 to 45 ^{0}C. Pre-incubation sample from bigeye snapper had no increase in breaking force when pre-incubation time was longer than 1 hour.

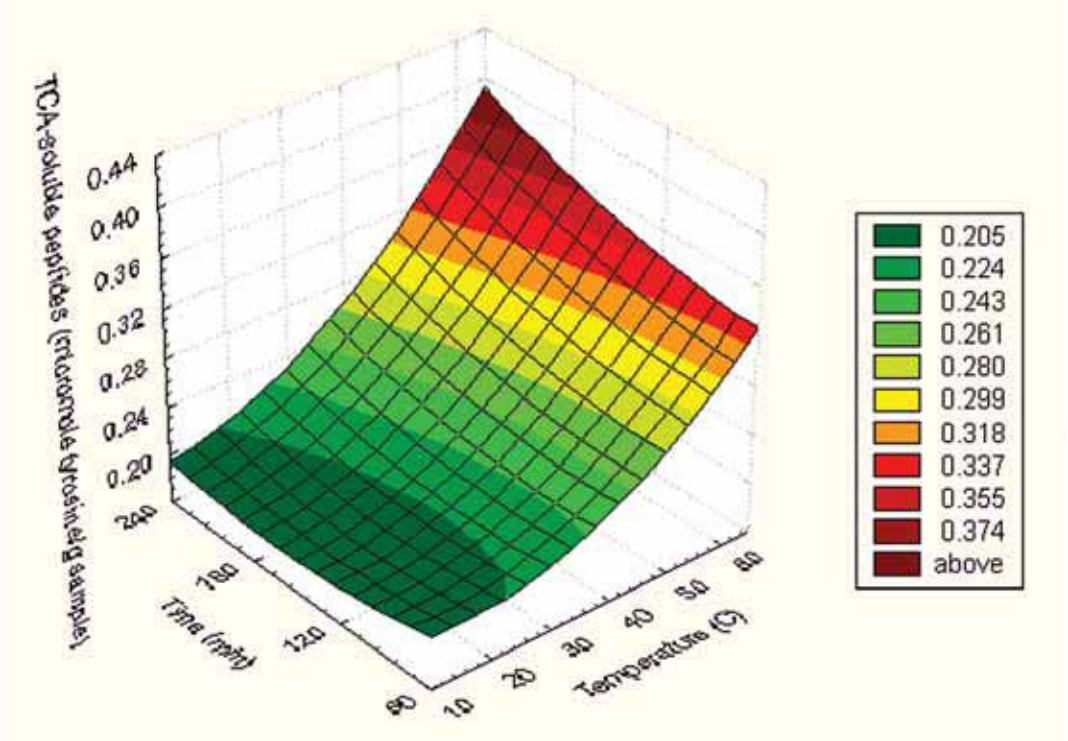

Figure 4 : Response surface plot for TCA-soluble peptides of cooked gels with different pre-incubated temperature and time

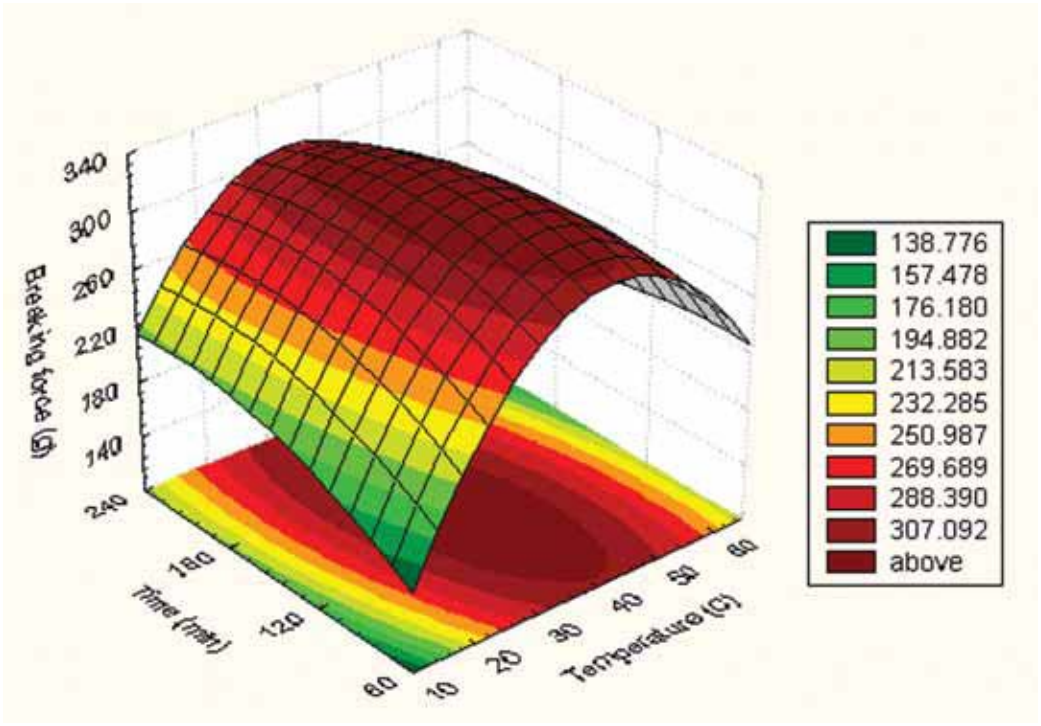

Figure 5 : Response surface plot for breaking force of cooked gels with different pre-incubated temperature and time

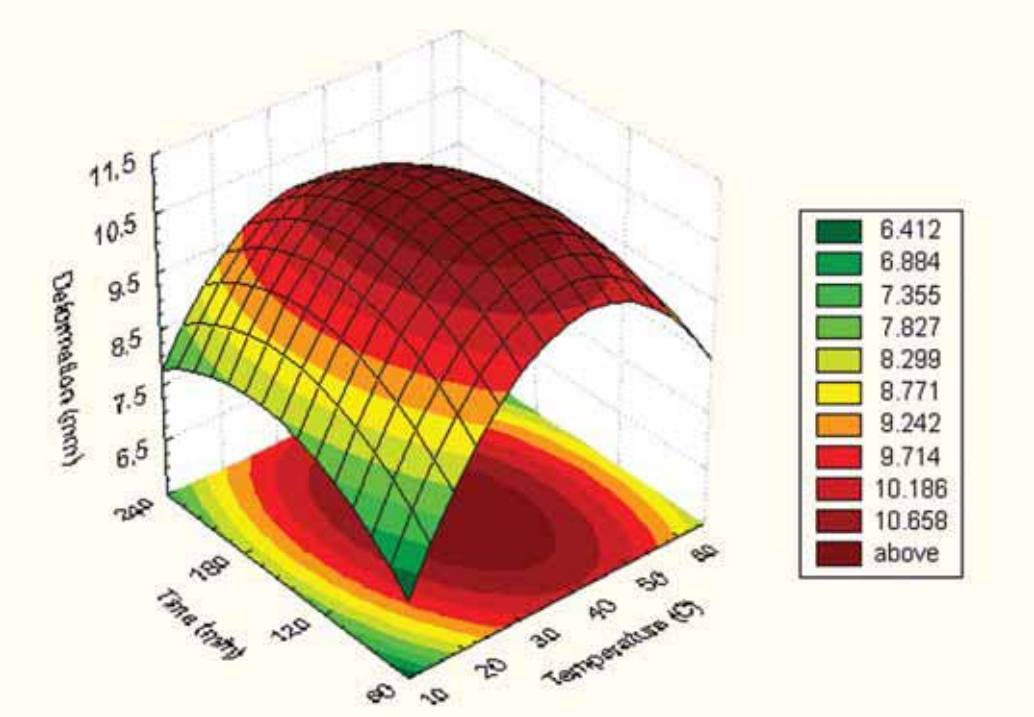

Figure 6 : Response surface plot for deformation of cooked gels with different pre-incubated temperature and time

According to Ferry (1948), proteins form gel networks through a coordinated transition from denaturation to gelation. It has been reported that the formation of a heat-induced gel requires the denaturation of the proteins. This process is accompanied by a conformational change and exposure of the reacting groups and followed by a second stage in which the denatured proteins establish protein to protein interactions, which leads toward aggregation (Luo, Pan, and Ji 2004). Sano, Noguchi, Marsumoto, and Tsuchiya (1990) suggested that the first stage of gel elasticity development was due to interactions among the tail portions of myosin molecules. The second stage was attributed to hydrophobic interactions among the head portions of myosin, because during heating the hydrophobic amino acids which are found mainly in the head portion exposed on the surface.

From the result of this study, the lower breaking force and deformation of cooked gel thanpre-incubation sample were noted. This result was possibly because the directly cooked gel was a rapid formation of disulfide and hydrophobic protein-protein bonds in the absence of the conditions required for the proteins to orient to form a network (Niwa 1985). Stronger gels can be obtained by pre-heating minced fish paste at temperature near 40 ^{0}C for short time or by refrigeration overnight prior to further heating (Luo and others 2001). Lanier, Lin, Hamann, and Thomas (1981) reported that the corresponding high level of soluble protein and a loss of extractable myosin in gels from minced Atlantic croaker processed at temperatures approaching the optimum for proteolytic activity (50 - 60 ^{0}C) would indicate that the loss in textural firmness was due to proteolytic degradation of the muscle proteins. At temperatures

above and below this region, proteolytic activity was decreased, and the textural firmness of gels was much greater.

Temperature plays an important role in fish paste gelation by affecting on the conformation of myofibrillar proteins. Temperature can activate endogenous enzymes that naturally occur in fish muscle (An and others 1996; Totosaus and others 2002). The different heating conditions for gel preparation caused different gelling properties. Gel quality of fish paste pre-incubated at temperature around 30 to 50 ^{0}C and above 70 ^{0}C was higher than at 60 ^{0}C. This possibly because proteolysis was occurred (Makinodan, Yamamoto, and Shimidu 1963; Deng 1981; Toyohara, Sakata, Yamashita, Kinoshita, and Shimizu 1990; Benjakul and others 2003). The strength of gel was initially increased during pre-incubation at temperature $\leq$ 40 ^{0}C before the samples were cooked. These phenomena strongly suggest that the pre-incubation process accelerates the cross-linking of myosin heavy chain via non-disulfide bond during the pre-incubation process due to endogenous transglutaminase (Boye and Lanier 1988; Numakura, Kimura, Toyoda, and Fujita 1990; Ho and others 2000; Hossain, Itoh, Morioka, and Obatake 2001; Luo and others 2001; Yongsawatdigul and others 2002; Benjakul and others 2003; Luo and others 2004; Uresti and others 2006). While, the textural properties of gels at different heating conditions exhibited the lowest breaking force and deformation in samples pre-incubated at 60 ^{0}C, it is probably due to the fact that at this temperature pre-incubation accelerated proteolytic activity and subsequently retarded gel network formation (Hossain and others 2001; Benjakul and others 2003; Yongsawatdigul and Piyadhammaviboon 2004). High temperature during heating led to further oxidation of sulphydryl groups with a subsequent disulfide bond formation (Niwa 1992; Benjakul and others 2003; Benjakul and others 2004). Hossain and others (2001) suggested that the polymerization by disulfide bonding occurs during cooking at 80 ^{0}C and not during pre-incubation.

From the results, the mathematical models expressing the correlation of pre-incubation temperature and time on the dependent variables of hybrid catfish gels showed significance in lack of fit. In addition, the pre-incubation time was found to have no influence on all the response parameters. On the other hand, the influence of the pre-incubation times on the dependent variables of surimi gels has been previously reported (Alvares and others 1999; Hossain and others 2001; Luo and others 2001; Benjakul and Visessanguan 2003; Benjakul and others 2003; Benjakul and others 2004; Luo and others 2004). This is probably due to the composition of fish mince similar to fish flesh that is abundant with other compositions such as sarcoplasmic proteins, lipid, pigment proteins and endogenous enzymes. There are factors that influence the fish gel forming ability; including high content of fat, sarcoplasmic proteins and proteinases (Kamal, Biswas, Yasmin, Azimuddin, and Nazrul Islam 2001). Viratchakul (2001) suggested that during heating process, the denatured sarcoplasmic proteins were binding with myofibrillar proteins, resulting in low gel forming ability. Endogenous serine proteinases in fish muscle are suspected to be responsible for the myosin degradation during thermal processing (Lanier and others 1981; Toyohara and others 1990; Ohkubo, Osatomi, Hara, Nozaki, Aranishi, and Ishihara 2004). Some degradation-inducing proteinases exist in the sarcoplasmic fraction. Washing processes have

been used to improve the quality of fish gel by a removal of sarcoplasmic protein, lipid, lysosomal proteinases and other water soluble compositions (Toyohara, Kinoshita, and Shimizu 1990; An, Weerasinghe, Seymour, and Morrissey 1994; Jiang, Lee, Tsao, and Lee 1997). In general, autolytic activity in washed mince was lower than mince, especially at the high temperature ranges. This might be due to a removal of sarcoplasmic proteinase during washing process (Benjakul and others 2003; Yongsawatdigul and Piyadhammaviboon 2004). Thus, the mathematical models expressing the correlation of pre-incubation temperature and time on the dependent variables of hybrid catfish minced gel were not adequate for prediction as well as the pre-incubation time has no influence on all the response parameters changes probably caused by the reason mentioned above.

Despite the inadequate of mathematical model, data showed that the actual value of breaking force and deformation of minced gel with different pre-incubated temperature and time showed significant effect of breaking force and deformation of minced gel ($P<0.05$) as shown in Table 8. The highest breaking force and deformation of minced gel were found in the sample pre-incubated at 45 ^{0}C for 150 minutes. Therefore, to produce a minced catfish gel with highest breaking force and deformation values the minced fish should be pre-incubated at 45 ^{0}C for 150 minutes followed by cooking at 90 ^{0}C for 30 minutes. However, to produce optimum quality minced gel further study to identify the optimum of breaking force and deformation values are recommended.

Table 8 : Breaking force and deformation of minced gel with different pre-incubated temperature and time *

Temperature (^{0}C)	Time (min)	Breaking force (g)	Deformation (mm)
45.0	22.7	259.3a	9.5 a
65.0	240.0	124.2 b	5.9 b
25.0	240.0	282.5c	9.2 c
25.0	60.0	254.2 d	9.0 d
45.0	277.3	311.4 e	10.7 e
73.3	150.0	236.3 f	8.9 f
16.7	150.0	242.0 g	9.2 c
65.0	60.0	202.6 h	7.9 g
45.0	150.0	315.6 i	11.0 h
45.0	150.0	319.9i	10.9 h
45.0	150.0	314.8 i	10.9 h
45.0	150.0	316.9 i	10.9 h
45.0	150.0	315.7 i	11.0 h

* Values are given as mean from three observations

CONCLUSION

Response surface methodology was used to investigate the effect of pre-incubation conditions on biochemical and textural properties changes of hybrid catfish minced gels. The results of this research indicated that changes in pre-incubation temperature affected the changes in response parameters. The estimated responses surfaces showed that an increasing of pre-incubation temperature influenced a decrease of pre-incubated samples and cooked gels solubility. When pre-incubation temperature was above 50 ^{0}C, an increasing in solubility of samples was observed. The highest in TCA-soluble peptides of pre-incubation samples and cooked gels were found at 65 ^{0}C. Breaking force and deformation of cooked gels were increased along with pre-incubation temperature increased. From the results, the improvement of the textural properties of minced hybrid catfish gel was achieved by pre-incubation at temperature 45 ^{0}C for 150 minutes before pre-incubated sample was cooked at 90 ^{0}C for 30 minutes.

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