Optimization of Gelatin Extraction from Thai Fish Panga (*Pangasius bocourti* Sauvage) Skin

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ABSTRACT

An investigation on optimal conditions for gelatin extraction from the Thai fish panga (Pangasius bocourti Sauvage) skin was performed by response surface methodology. A Box-Behnken design was applied to examine the effects of extraction temperature (40-70°C), pH (3.7-7.4) and extraction time (1-5 h) on gelatin yield, gel strength and gel colour. All regression models were significant (P≤0.01) and lack-of-fit of the models was insignificant, except for that of the gel strength. The Anderson-Darling normality test of the standardized residuals showed adequacy of all models. The optimal conditions for gelatin extraction were at 55°C, pH 4.55 for 1 h. The predicted responses were 20.22% gelatin yield, 506.55 g gel strength, 42.22 lightness (L*), 3.56 chroma (C*) and 43.35° hue angle (h°). The experimental responses of gelatin extracted at the optimal conditions were not significantly different (P>0.5) from the predicted value.

Key words: Gelatin, Thai fish panga, Response surface methodology, Physical properties

INTRODUCTION

Gelatin is a biopolymer obtained from partial hydrolysis of collagen. It has been used in many fields such as food, pharmaceutical, photographic and cosmetic industries. In food industry, it has been used as a gelling agent and an edible film. Gelatin can also promote healthy bones, joints and skin (Kasankala et al., 2007; Rahman et al., 2008).

Gelatin was previously extracted from bovine or swine skin or bones. However, since bovine spongiform encephalopathy (BSE) and foot-and-mouth disease had occurred, consumer became hesitant to eat food derived from these terrestrial animals. Fish are then an alternative source for gelatin production. Although it was reported that the bloom strength of fish gelatin was lower than that of bovine or swine gelatin, pretreatment of skin with saline or hydrogen peroxide solution could increase the bloom strength of fish gelatin (Giménez et al., 2005; Aewsiri et al., 2009).

The Thai fish panga (*Pangasius bocourti* Sauvage) is a new economic fish that has been promoted to be cultured in areas along the Mae Khong shore

of Thailand. The fish is processed to frozen fillets for export to Europe and the USA. In the processing, many parts of the fish, such as skin and bones, are usually discarded (National Food Institute, 2006). However, the skin is composed of high amounts of collagen that can be converted to gelatin. Accordingly, the value is added to the skin by-products, and disposal problem is also diminished.

Our preliminary study found that pretreatment of fish skin with 0.8 M sodium chloride in 0.1 M sodium hydroxide solution resulted in increasing gelatin yield and gel strength compared with pretreatment with sodium hydroxide solution alone or hydrogen peroxide in sodium hydroxide solution. The principal objective of this study was to investigate an optimal condition for extraction of gelatin from the Thai fish panga skin pretreated with 0.8 M sodium chloride in 0.1 M sodium hydroxide solution, using acetic acid to adjust the pH. Gelatin yield, gel strength and gel colour were determined at various extraction temperatures (40-70°C), pH levels (3.7-7.4) and lengths of extraction time (1-5 h).

MATERIALS AND METHODS

Raw materials

The frozen Thai fish panga skin was obtained from a processing plant at Nakhonphanom province of Thailand and kept at -20°C prior to use. Proximate composition of the skin was 60.86% moisture, 35.83% crude protein, 2.19% crude lipid and 0.18% crude ash.

Reagents

Extraction chemicals included sodium hydroxide (Merck, Germany), sodium chloride (Union Science, Thailand) and glacial acetic acid (Labscan, Thailand). Analytical reagents included cupric sulfate 5-hydrate (J.T. Baker, USA), potassium sodium tartrate (Univar, Australia) and bovine serum albumin (Sigma-Aldrich, Canada).

Fish skin pretreatment

Fish skin was manually scraped off the flesh. The skin was then cut into the square dimension with the size of 1-2 cm. The fish skin was pretreated by stirring for 4 h in a solution of 0.8 M sodium chloride and 0.1 M sodium hydroxide at a skin-per-solution ratio of 1:20 (w/v). The solution was changed after 2 h of use. The pretreated skin was then rinsed 3 times with water before extraction with various concentrations of acetic acid solution.

Experimental design

The optimal condition for processing gelatin from the Thai fish Panga was determined by the response surface methodology. The Box-Behnken design was used to examine the effects of 3 independent variables—extraction temperature, pH and extraction time—on gelatin yield, gel strength and gel colour. The symbols and levels of independent variables are shown in Table 1. Five replicates at the central point of the designed model were used to estimate the pure error sum of squares.

Table 1. Experimental design range and levels of the independent variables for the production of the Thai fish panga skin gelatin.

Independent variables	Symbol	Range and levels		
Coded value:		-1	0	1
Real value:				
Temperature (°C)	X_1	40	55	70
pH	X_2	3.7	5.55	7.4
Time (hours)	X_3	1	3	5

Gelatin extraction

The pretreated skin was extracted by 17 treatments (Table 2). The pH of the extracting solution was adjusted to 3.70, 5.55 or 7.40 using glacial acetic acid. The pretreated skin was suspended in the extracting solution with the sample-per-solution ratio of 1:6 (w/v) (Kołodziejska et al., 2008). Temperatures of the mixture were controlled at 40, 55 and 70°C using hot water bath. After extraction, the mixture was filtered through a piece of double-layer cheese cloth and then centrifuged at 2,000 g for 30 min to obtain gelatin solution as a supernatant. The protein content in the supernatant was determined. The gelatin solution was dried out overnight, using forced air oven at 50°C to obtain gelatin sheets with 13-14% moisture content. The dried gelatin sheets were measured for gel strength and colour.

Table 2. Experimental and predicted values of gelatin yields and gel strength responses of the gelatin extracted from the Thai fish panga.

Standard order	Independent variables[1]			Gelatin yield (%)		Gel strength (g)	
	Temperature (°C)	pH	Time (h)	Experimental value	Predicted value	Experimental value	Predicted value
1	-1 (40)	-1 (3.70)	0 (3)	19.12±1.26	18.11	463.49±4.53	476.51
2	+1 (70)	-1 (3.70)	0 (3)	21.36±1.15	21.35	445.68±4.66	455.62
3	-1 (40)	+1 (7.40)	0 (3)	0.35±0.01	0.36	-	498.15
4	+1 (70)	+1 (7.40)	0 (3)	20.90±0.93	21.91	397.45±9.82	384.37
5	-1 (40)	0 (5.55)	-1 (1)	3.35±0.07	3.79	587.06±8.44	552.10
6	+1 (70)	0 (5.55)	-1 (1)	20.45±1.48	19.89	495.59±10.31	479.76
7	-1 (40)	0 (5.55)	+1 (5)	11.50±1.28	12.06	486.43±13.78	485.68
8	+1 (70)	0 (5.55)	+1 (5)	21.19±0.46	20.75	407.06±7.72	413.34
9	0 (55)	-1 (3.70)	-1 (1)	20.33±0.62	20.91	479.69±12.67	494.28
10	0 (55)	+1 (7.40)	-1 (1)	8.05±0.30	7.60	460.84±3.86	474.47
11	0 (55)	-1 (3.70)	+1 (5)	20.32±0.95	20.76	455.42±5.49	427.86
12	0 (55)	+1 (7.40)	+1 (5)	17.46±0.97	16.89	408.60±6.03	408.05
13	0 (55)	0 (5.55)	0 (3)	22.10±1.63	21.40	473.32±2.30	482.72
14	0 (55)	0 (5.55)	0 (3)	22.38±1.33	21.40	479.77±7.34	482.72
15	0 (55)	0 (5.55)	0 (3)	21.32±0.75	21.40	464.03±6.26	482.72
16	0 (55)	0 (5.55)	0 (3)	20.41±0.68	21.40	481.70±5.03	482.72
17	0 (55)	0 (5.55)	0 (3)	20.79±0.60	21.40	469.52±6.77	482.72

[1]Numbers outside parentheses are coded values; numbers in parentheses are actual values.

Table 3. Experimental and predicted values of color responses of the gelatin extracted from the Thai fish panga.

Standard order	Independent variables[1]			Lightness (L*)		Chroma (C*)		Hue angle (h°)	
	Temperature (°C)	pH	Time (h)	Experimental value	Predicted value	Experimental value	Predicted value	Experimental value	Predicted value
1	-1 (40)	-1 (3.70)	0 (3)	45.62±0.75	45.51	2.77±0.02	2.68	33.52±2.85	34.71
2	+1 (70)	-1 (3.70)	0 (3)	42.00±1.50	42.47	4.70±0.27	4.72	54.75±2.57	55.14
3	-1 (40)	+1 (7.40)	0 (3)	-	35.97	-	4.71	-	19.86
4	+1 (70)	+1 (7.40)	0 (3)	35.88±1.22	36.46	5.15±0.39	5.24	41.86±5.74	40.28
5	-1 (40)	0 (5.55)	-1 (1)	41.30±1.27	42.24	3.11±0.03	3.09	36.64±3.73	35.13
6	+1 (70)	0 (5.55)	-1 (1)	41.33±0.45	40.96	3.86±0.23	3.74	46.33±0.46	46.01
7	-1 (40)	0 (5.55)	+1 (5)	44.11±0.91	43.28	2.99±0.08	3.09	34.23±4.31	34.55
8	+1 (70)	0 (5.55)	+1 (5)	42.69±1.90	42.01	5.00±0.51	5.00	63.01±3.48	64.52
9	0 (55)	-1 (3.70)	-1 (1)	44.03±1.99	43.33	2.92±0.06	3.04	34.51±7.29	34.64
10	0 (55)	+1 (7.40)	-1 (1)	33.40±0.05	32.59	4.31±0.22	4.32	11.11±2.78	12.81
11	0 (55)	-1 (3.70)	+1 (5)	41.06±1.46	41.40	3.69±0.17	3.67	38.33±0.46	36.63
12	0 (55)	+1 (7.40)	+1 (5)	36.37±0.82	36.60	5.02±0.33	4.95	28.87±3.54	28.75
13	0 (55)	0 (5.55)	0 (3)	40.26±1.14	40.50	4.05±0.51	4.60	50.89±4.66	48.49
14	0 (55)	0 (5.55)	0 (3)	41.21±0.17	40.50	4.01±0.65	4.60	52.43±3.47	48.49
15	0 (55)	0 (5.55)	0 (3)	40.10±2.12	40.50	5.06±0.14	4.60	50.46±2.76	48.49
16	0 (55)	0 (5.55)	0 (3)	39.89±1.35	40.50	5.30±0.24	4.60	46.75±4.61	48.49
17	0 (55)	0 (5.55)	0 (3)	40.09±0.23	40.50	4.61±0.20	4.60	41.91±0.53	48.49

[1]Numbers outside parentheses are coded values; numbers in parentheses are actual values.

Gelatin yield determination

The protein content of the gelatin solution was determined by the Biuret method (Weaver and Daniel, 2003). In brief, 100 ?l of the sample was mixed with 300 µl water and 1.6 ml Biuret reagent (0.15% copper sulfate and 0.6 sodium potassium tartrate in 3% sodium hydroxide solution). The solution was then kept for 30 min at room temperature before measuring the optical density at 550 nm, using bovine serum albumin as the standard. The gelatin yield was calculated as follows:

Yield (%) = protein content in supernatant (g) × 100 / weight of fish skin used (g)

Gel strength determination

Gel strength was analyzed using to the method of Zhou and Regenstein (2004). Gelatin solution of 6.67% (w/w) was prepared by dissolving dried gelatin with distilled water and heated at 60±1°C for 30 min in a water bath. After that, the gelatin solution was filled in a cup (30 mm diameter × 15 mm height) and kept at 2±0.4°C for 16-18 h. The gel strength was measured by the texture analyzer (TA.XT Plus, Stable Micro System, England), using a 12.7 mm diameter plunger (P/0.5R probe), 0.5 mm/s compression rate and 4 mm penetration depth. The gel strength is a maximum force required in penetration.

Color measurement

The 6.67% gelatin solution was prepared as described above and measured for the color in L*C*h° scale, using Minolta Chroma Meter, CR300 model (Minolta, Japan).

Regression models

The response surface regression was analyzed, using the Design Expert software (Stat-Ease, Inc., USA). The following quadratic polynomial equation was a proposed regression model,

$$Y_i = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

where Yi were the dependent variables, β_0 was a constant, β_i, β_{ii}, β_{ij} were the regression coefficients and X_i, X_j were the independent variables. Some terms were excluded in this analysis to make the significant regression model with insignificant lack-of-fit, which has high correlation coefficient (R^2). The optimal extraction condition that resulted in high gelatin yield, gel strength and lightness was obtained from the models.

The Anderson-Darling normality test was used to evaluate the adequacy of the model by plotting between the standardized residual (difference between the observed value and the predicted value divided by its standard deviation) of the dependent variables and their correspondence probabilities (Cho et al., 2005). The Minitab software (Minitab, Inc., State College, Pa, U.S.A.) was used in this analysis.

Model verification

Gelatin was extracted in triplicate using the obtained optimal conditions. Analysis of variance was carried out to test the difference between the experimental and the predicted optimal responses (Cho et al., 2005). A statistical analysis of this step was performed by the Minitab software.

RESULTS AND DISCUSSION

Response model

The experimental data are shown in Table 2. The physical properties of the gelatin extracted by treatment 3 were not evaluated because the gelatin yield was too low. The coefficients of independent variables, P-value and R^2 of the models, are shown in Table 4. All regression models were highly significant (P<0.01) and the lack-of-fit was insignificant (P>0.5), except for that of the gel strength.

Table 4. Coefficients of coded and uncoded independent variables with *P*-value and R^2 of models.

Model details	Gelatin yield (%)	Gel strength (g)	Color		
			L*	C*	h°
Coefficient of real value					
k	-20.352	182.871	73.763	-7.437	-31.667
Temp	1.732	1.734	-1.013	0.215	-1.228
pH	-7.553	138.071	1.493	1.092	38.297
Time	5.631	-16.604	-1.968	0.492	-2.196
$Temp^2$	-0.019		0.007	-0.001	0.013
pH^2	-0.518	-9.220	-0.590		-4.067
$Time^2$	-0.772			-0.152	-1.590
Temp × pH	0.165	-0.747	0.032	-0.014	
Temp × Time	-0.062			0.011	0.159
pH × Time	0.637		0.401		0.942
Coefficient of coded value					
β_0	21.400	482.721	40.500	4.601	48.486
X_1	6.198	-36.169	-0.637	0.641	10.213
X_2	-4.296	-9.904	-3.888	0.639	-7.428
X_3	2.286	-33.209	0.521	0.313	4.481
X^2_1	-4.192		1.623	-0.262	2.929
X^2_2	-1.774	-31.557	-2.020		-13.918
X^2_3	-3.086			-0.609	-6.361
X_1X_2	4.578	-20.725	0.884	-0.377	
X_1X_3	-1.852				4.772
X_2X_3	2.358		1.485	0.315	3.487
P-value					
Model	<0.0001	0.0003	<0.0001	0.0044	0.0003
Lack of fit	0.2618	0.0189	0.1065	0.9943	0.8428
Adjusted R^2 (%)	97.92	80.99	93.41	76.62	91.83

Gelatin yield

The response model for gelatin yield was

$$Y_1 = 21.400 + 6.198X_1 - 4.296X_2 + 2.286X_3 - 4.192X_1^2 - 1.774X_2^2 - 3.086X_3^2 + 4.578X_1X_2 - 1.852X_1X_3 + 2.358X_2X_3$$

All terms were significant at 99% confidence level. The adjusted correlation coefficient of the model (R^2) was 97.92%.

The gelatin yield increased with the rise of extraction temperature and the decrease of pH solution (Fig.1). This is because gelatin is well soluble in acid solution and the solubility is promoted by a high temperature (O'Neil et al., 2001). At a low temperature, collagen could be extracted and solubilized without altering its triple-helix configuration. At a high temperature, however, both hydrogen and covalent bonds are cleaved, the triple-helix configuration is destabilized and the helix-to-coil transition occurs (Montero and Gómez-Guillén, 2000). This phenomenon makes the solubilization of gelatin easier.

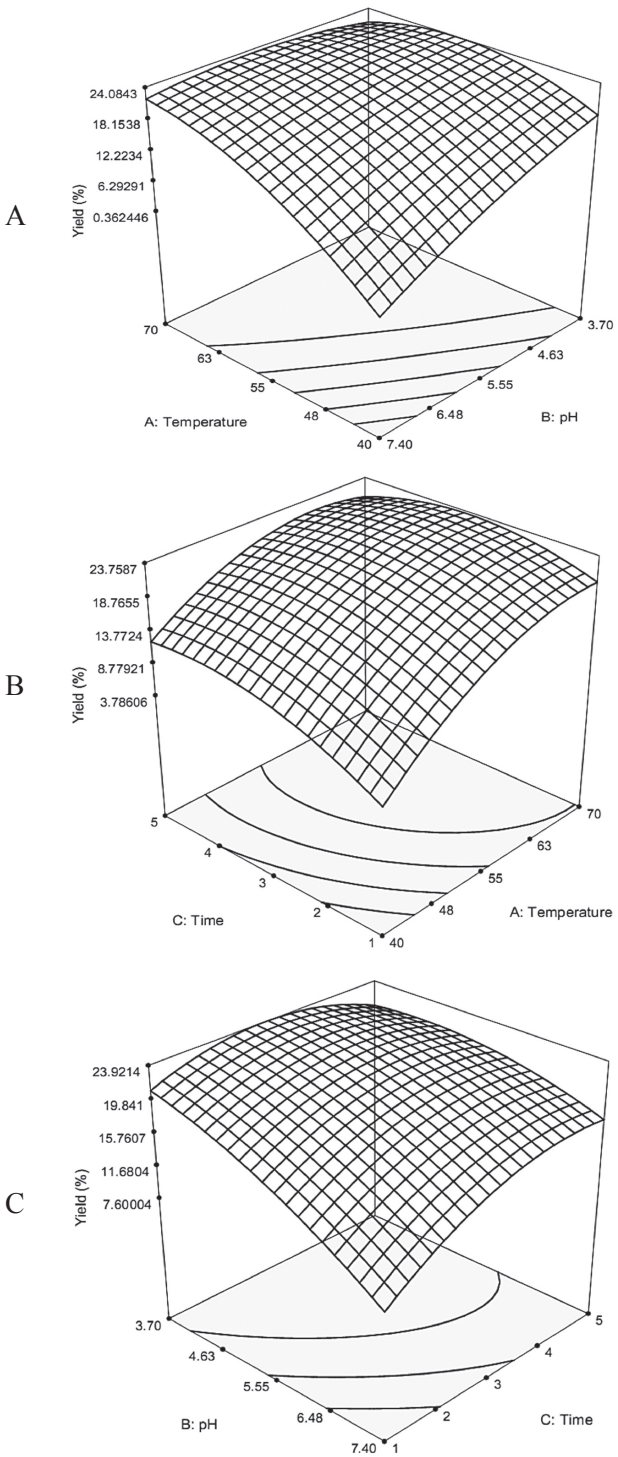


Figure 1. Response surface of gelatin yield (%) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)

The gelatin yield also increased with the increase of extraction time. However, extraction for too long at low pH resulted in reduction of gelatin yield. The similar pattern was reported by Cho et al., (2006). According to the coefficient of model terms (Table 4), the extraction temperature was the main effect on the response, when compared with the pH level and extraction time.

Gel strength

The response model for gel strength was

$$Y_2 = 482.721 - 36.169X_1 - 9.904X_2 - 33.209X_3 - 31.557X_2^2 - 20.725X_1X_2$$

All terms were significant ($P \leq 0.05$), except for the values of X_2 and X_1X_2, but they would be accounted for in the model to provide the high correlation coefficient (80.99%). The lack-of-fit ($P \leq 0.05$) of the model indicated that the quadratic model may not be suitable for explaining the behavior of gel strength as a function of the three factors. This result agreed with the result of Yang et al., (2007), that the gel strength of gelatin extracted from the channel catfish skin could be predicted by neither the quadratic nor the linear model.

The gel strength decreased with the increase of temperature and extraction time (Fig. 2). Although gelatin can be extracted more easily at a higher temperature and with a longer treatment time, this severe condition would break the bonding and result in the release of free amino acid that causes reduction of gel strength (Cho et al., 2006). The maximum gel strength was observed at a pH level between 4.5 and 5.5, depending on the extraction temperature and time. From the results in Fig. 2A and Fig. 2C, extraction at pH lower than 4.5 would cause acid hydrolysis of gelatin molecules that results in the decrease of gel strength. Zhou and Regenstein (2005) reported that gelatin extracted from the Alaska pollock skin had the highest gel strength when extracted at pH 6, and that the gel strength decreased when the pH was lower or higher. The deviation of the results may come from the design points. This study was designed at pH 3.70, 5.55 and 7.40, while that of Zhou and Regenstein (2005) was designed at pH between approximately 3-9.

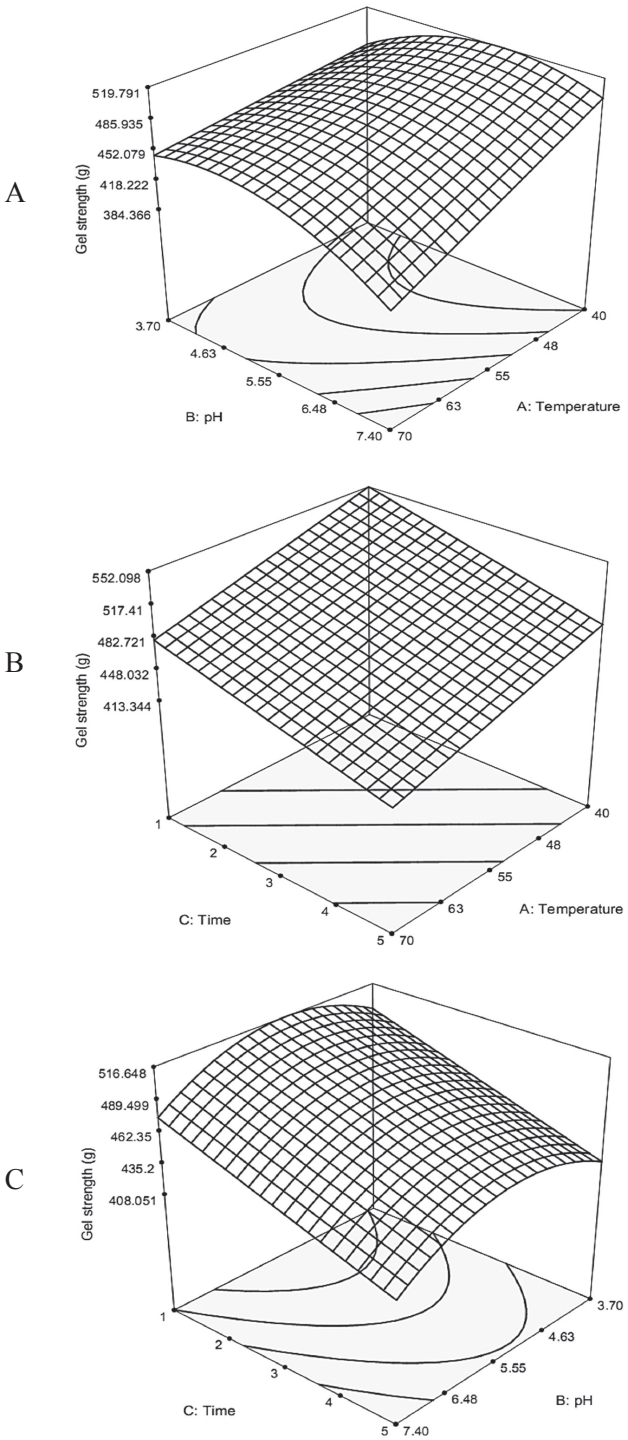


Figure 2. Response surface of gel strength (g) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)

Colour

Gel colour is another factor that has been widely used to determine the physical quality of gelatin. The quadratic models of all colour responses were significant (P<0.01), with insignificant lack-of-fit (P>0.5). Table 4 presents the adjusted R^2 of the colour responses which were acceptable for the prediction of the responses. Lightness (L*) of the gelatin was highly affected by pH (Table 4). Extraction of gelatin at higher pH caused gelatin to become darker (Fig. 3). Chroma (C*) is used to describe the colour saturation of the objects. If the chroma value equal 0, the object color was white, grey or black depending on L*. The more chroma value, the object became more colourful (Cruse, 2009). The chroma of the gelatin ranged between 2 to 5, which suggested that gel color was pale. The regression model of the chroma had many insignificant terms (X_3, X^2_1, X_1X_2 and X_1X_3) but these terms produced higher correlation coefficients, so these terms were accounted for in the model. According to the model, temperature and pH were the main effects on the chroma. Extraction at a high temperature and high pH caused the colour of the gelatin to have higher intensity (Fig 4). Temparature also proved to have a major influence on hue angle (h°). Extraction at a high temperature caused gel color to change from pink to yellow (Fig. 5). Nevertheless, since the chroma value was quite low, variation of gel color or hue angle may not be visually observable. Thus, chroma and hue angle may not be deemed as the important factor to determine the quality of gelatin extracted by conditions used in this study.

Normality test

Normal probability plots of the standardized residuals are shown in Fig. 6 and Fig. 7. The standardized residuals greater than 2 and smaller than -2 are usually considered as large. The gelatin yield had two large residuals (Fig. 6A) while the gel strength, lightness and hue angle had one large residual (Fig. 6B, 7A and 7C, respectively), and chroma had no large residual (Fig 7B). According to the Anderson-Darling normality test, the standardized residuals of all responses had the normal distribution (P>0.5), indicating the adequacy of the models.

The distribution of gel strength's residual was nearly significant (P=0.064). This result confirmed a significant model with lack-of-fit. Cho et al., (2005) also reported a similar pattern, that the quadratic model of gel strength had both significant model and lack-of-fit, although its residuals were distributed normally.

Optimal condition

In commercial production, the main purpose for extracting gelatin is to obtain gelatin with high yield. The gelatin should also have high gel strength and light color. Therefore, yield, gel strength, lightness and temperature were used in prediction of an optimal condition for the extraction of gelatin (Table 5). The optimal condition was extraction at 55°C for 1 h at pH 4.55, for which the predicted responses would be 20.22% gelatin yield, 506.55 g gel strength, 42.22 lightness (L*), 3.56 chroma (C*) and 43.35? hue angle (h°).

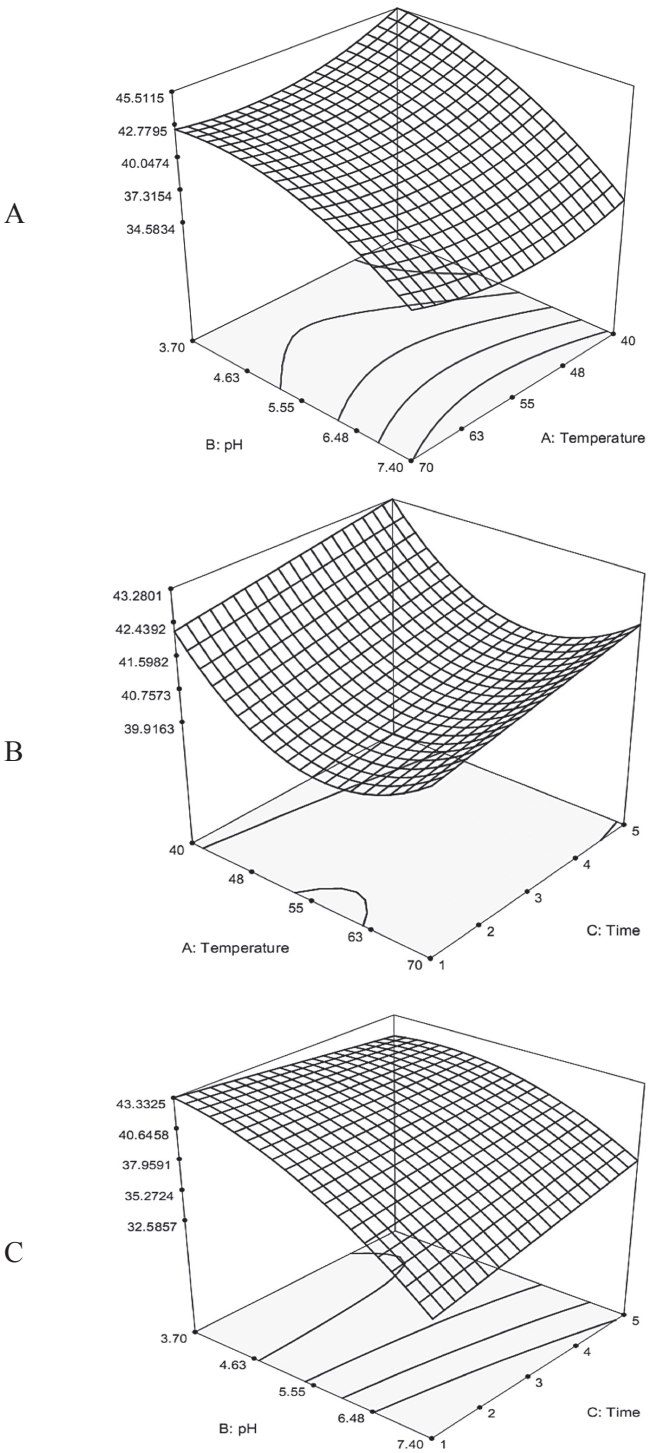


Figure 3. Response surface of lightness (L*) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)

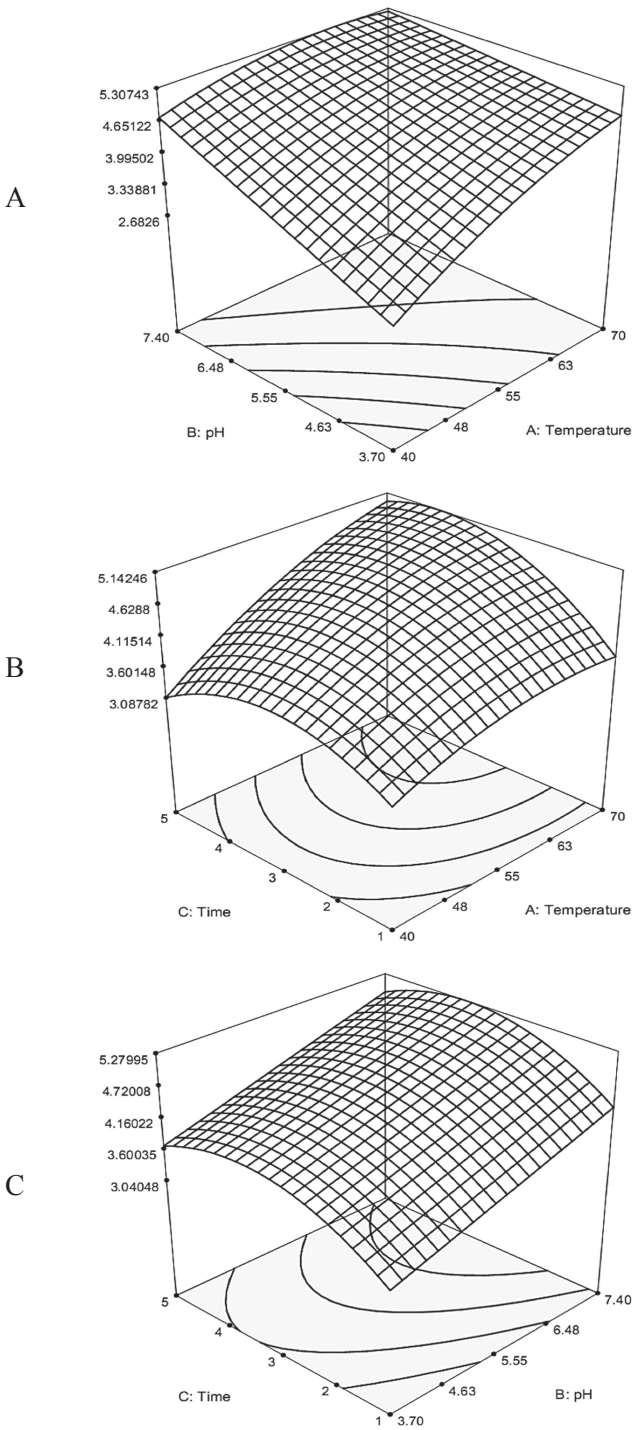


Figure 4. Response surface of chroma (C*) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)

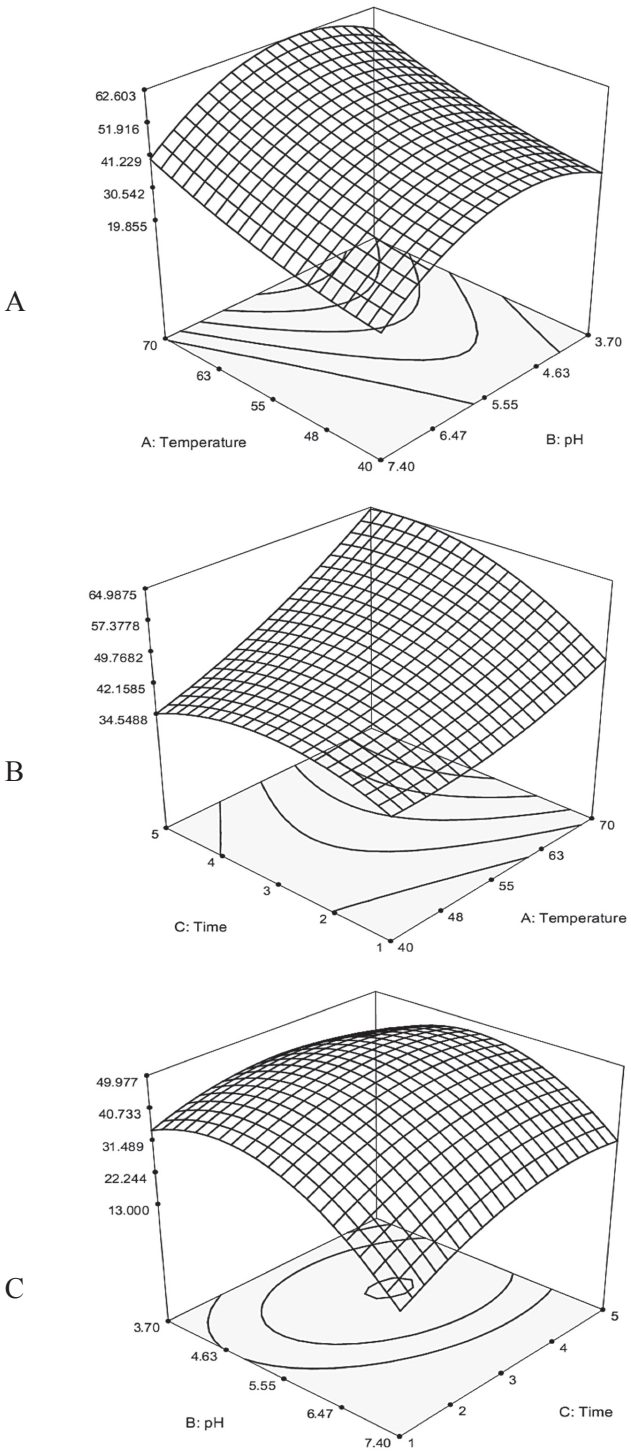
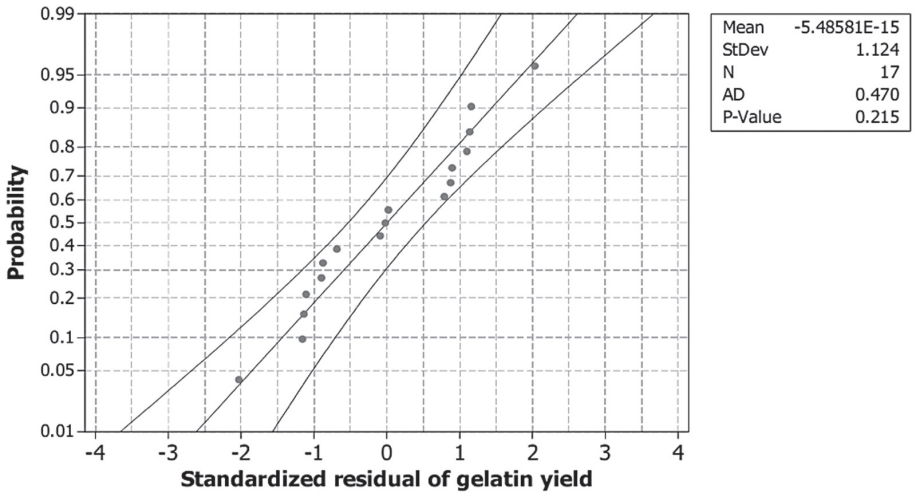


Figure 5. Response surface of hue angle (h°) as a function of (A) extraction temperature and pH, (B) temperature and time, and (C) pH and time. (The third factor in each graph was fixed at the mid point.)

A



B

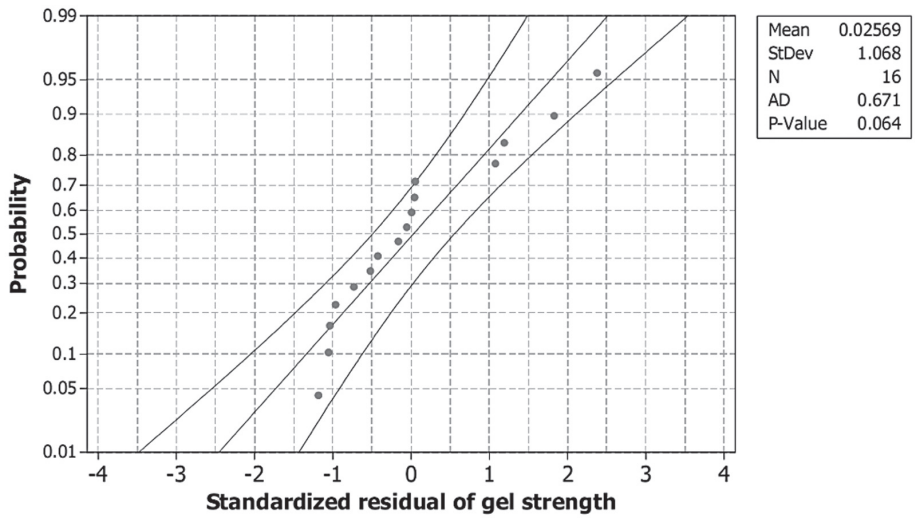
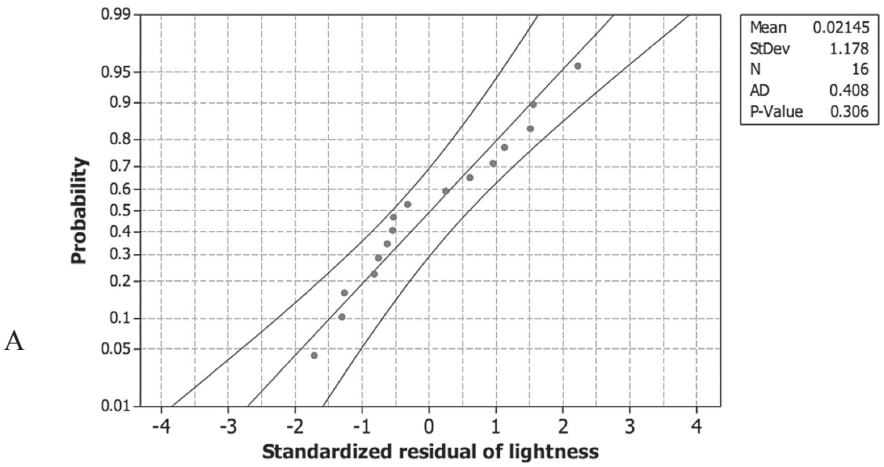
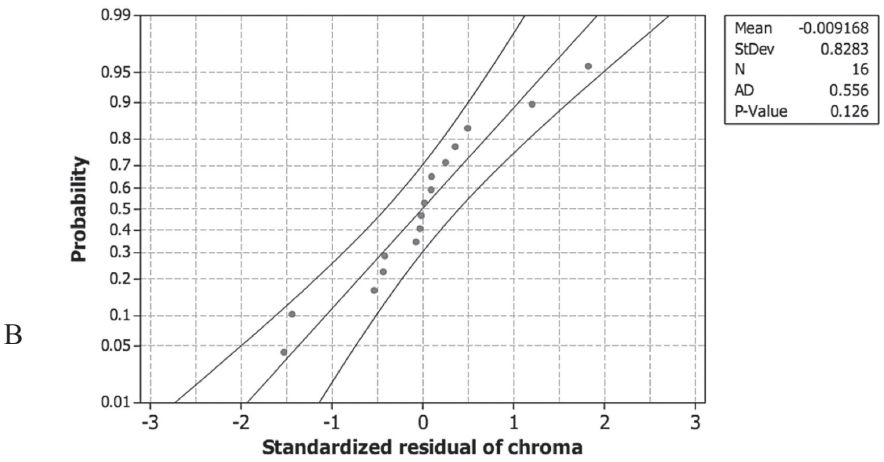


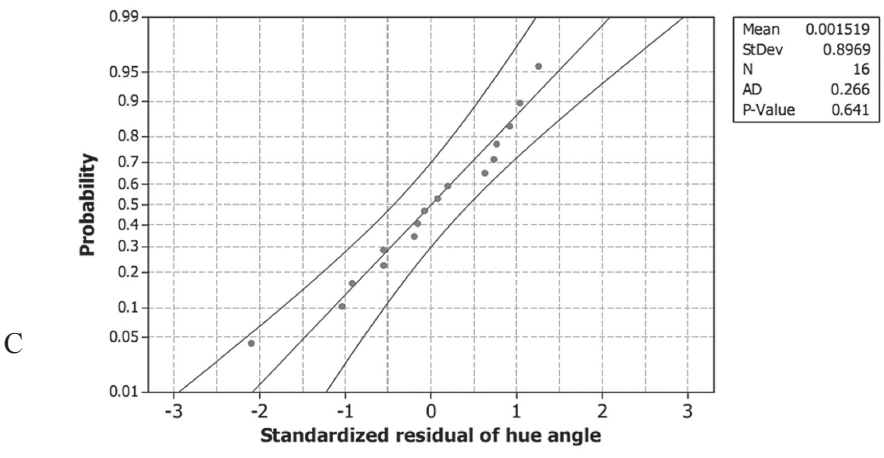
Figure 6. Normal probability plots for error terms using standardized residuals of gelatin yield (A) and gel strength (B), based on the Anderson-Darling normality test.



A



B



C

Figure 7. Normal probability plots for error terms using standardized residuals of lightness (A), chroma (B) and hue angle (C), based on the Anderson-Darling normality test.

Table 5. Optimization parameters used in Design Expert software.

Criteria	Goal	Lower	Upper	Weight	Importance
Temperature (°C)	Minimum	40	70	1	+
Yield (%)	Maximum	19	22.4	1	+++
Gel strength (g)	Maximum	500	587	1	+++
Lightness (L*)	Maximum	33.4	45.6	1	+

Cho et al., (2005) reported that an optimal condition for the extraction of gelatin from the yellowfin tuna skin was at 58.15°C for 4.72 h at pH of 6.0. The temperature reported by Cho et al., (2005) was close to this study, but their extraction time was much longer than that in this study. Liu et al., (2008) reported that an optimal condition for the extraction of gelatin from the channel catfish skin was extraction in 43.2°C water for 5.73 h at neutral pH. Kasankala et al., (2007) reported that the optimal conditions for gelatin extraction from the grass carp skin, pretreated for 24 h in 1.19% HCl solution, was at 52.61°C for 5.12 h. The discrepancy between this study and the previous studies may be mainly due to the difference in raw material and the pH used.

Model verification

The predicted and experimental responses of gelatin extracted at optimal conditions are shown in Table 6. The differences between the predicted and the experimental responses were insignificant ($P>0.5$), indicating that the regression models were suitable for the prediction of the studied responses.

Table 6. Experimental and predicted responses of the gelatin extracted at the optimal condition.

Dependent variables	Predicted value	Experimental value
Gelatin yield (%)	20.22	20.45±1.28
Gel strength (g)	506.55	508.22±22.05
Lightness (L*)	42.22	43.74±1.86
Chroma (C*)	3.56	3.31±0.61
Hue angle (h°)	43.35	42.68±4.71

CONCLUSION

The quadratic models as functions of extraction temperature, pH and time were suitable for the prediction of gelatin yield and gel colour. Although gel strength model had lack-of-fit, the results from normality test and model verification indicated that the gel strength model could be used to predict gel strength. The optimal condition for the extraction of gelatin from the skin of the Thai fish panga was at 55°C for 1 h at pH 4.55. The predicted responses from the optimal condition were 20.22% gelatin yield, 506.55 g gel strength, 42.22 lightness (L*), 3.56 chroma (C*) and 43.35° hue angle (h°). All values obtained from the experimental responses were in accordance with the predicted values.

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