Optimization and physical properties of gelatin extracted from pangasius catfish (*Pangasius sutchi*) bone

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Abstract In the present study, to establish the optimum gelatin extraction conditions from pangasius catfish (*Pangasius sutchi*) bone, Response Surface Methodology (RSM) with a 4-factor, 5-level Central Composite Design (CCD) was conducted. The model equation was proposed with regard to the effects of HCl concentration (%), treatment time (h), extraction temperature (°C) and extraction time (h) as independent variables on the hydroxyproline recovery (%) as dependent variable. $X_1=2.74\%$, $X_2=21.15\text{ h}$, $X_3=74.73\text{ °C}$ and $X_4=5.26\text{ h}$ were found to be the optimum conditions to obtain the highest hydroxyproline recovery (68.75 %). The properties of optimized catfish bone gelatin were characterized by amino acid analysis, SDS-PAGE, gel strength, TPA and viscosity in comparison to bovine skin gelatin. The result of SDS-PAGE revealed that pangasius catfish bone gelatin consisted of at least 2 different polypeptides (α_1 and α_2 chains) and their cross-linked chains. Moreover, the pangasius catfish bone gelatin was found to contain 17.37 (g/100 g) imino acids (proline and hydroxyproline). Pangasius catfish bone gelatin also indicated physical properties comparable with that of bovine and higher than those from cold water fish gelatin. Based on the results of the present study, there is a potential

for exploitation of pangasius catfish bone for gelatin production. Furthermore, RSM provided the best method for optimizing the gelatin extraction parameters.

Keywords Gelatin · Optimization · Response Surface Methodology · *Pangasius catfish* · Physical properties

Introduction

Collagen is an abundant protein in animal tissues and constitutes approximately 30 % of total animal protein. It is widely distributed in skin, bone, cartilage, tendons and all other organs of vertebrates (Sikorski 2001). Gelatin is a mixture of peptides and proteins produced by partial hydrolysis of collagen. It is one of the most popular biopolymers and widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties (Karim and Bhat 2009; Schrieber and Gareis 2007). The worldwide production amount of gelatin is about 326,000 t per year and its global demand has been increasing over the year (GME 2008).

On a commercial scale, gelatin is produced from mammalian by-products. According to Gudmundsson and Hafsteinsson (1997), bovine gelatin has a potential risk of spreading bovine spongiform encephalopathy (BSE). However, the Food and Drug Administration (FDA) has been monitoring the potential risk of animal-derived products since 1997 and concluded that there is very low or no risk of transferring prions through gelatin (Schrieber and Gareis 2007). On the other hand, gelatin produced from porcine is unacceptable for both Muslims and Jews for religious reasons, while Hindus do not consume cow-related products (Wangtueai and Noomhorm 2009). Therefore, the search for alternative sources has been initiated and accelerated.

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The seafood processing usually leads to enormous amounts of waste such as skin, bone and scale with high collagen content thereby promising alternative materials for gelatin extraction (Zhou et al. 2006; Yang et al. 2007). Bones are mineralized material with collagen fibril as the basic building block. Native collagen or gelatin from bone after previous demineralization can be obtained and such collagen is called ossein (Skierka et al. 2007). Since the presence of minerals in bone impairs the physicochemical properties of collagen preparation, it is possible to obtain pure collagen from bone only after removing minerals (Zelechowska et al. 2010). Therefore, one of the most important steps to prepare collagen for a successful gelatin extraction from bone is demineralization. In general, gelatin extraction process consists of two main stages: pre-treatment of the raw material and hot water extraction of gelatin. The length of the polypeptide chains, the degree of conversion of collagen into gelatin and the properties of the gelatin are related to both the pre-treatment and the extraction process (Gómez-Guillén et al. 2011). An acid pre-treatment for demineralization followed by an alkaline neutralization can provide a neutral extraction medium, which may resulted in a high gelatin yield with desirable gelling property (Zhou and Regenstein 2005; Zelechowska et al. 2010).

The quality of gelatin depends on its physicochemical properties, which are greatly influenced, not only by the origin of raw material, but also by the processing method and parameters (Cheow et al. 2007). The main problem of fish gelatin is that their gels tend to be less stable and have poorer gelling properties than gelatin from mammals, and this may limit their application. Based on the previous studies, this is true in the case of cold-water fish species, such as cod, salmon and alaska pollock. However, researchers have pointed out that gelatin from tropical and sub-tropical warm-water fish species (tilapia, Nile perch, catfish) might have similar physicochemical properties to that of mammalian, depending on the species, type of raw material and processing conditions (Gilsenan and Ross-Murphy 2000; Gómez-Guillén et al. 2009; Jamilah and Harvinder 2002; Muyonga et al. 2004; Rawdkuen et al. 2010).

Research on fish gelatin is important for the development of methods to produce gelatin from fish processing as replacements for mammalian sources. Scale of production and quality of gelatin depend on extraction method (Montero and Gómez-Guillén 2000). Optimization is the best suitable technique for this purpose. Recently, response surface methodology (RSM) has been used to evaluate the effectiveness of food manufacturing processes, including optimization of gelatin extraction (Wangtueai and Noomhorm 2009). RSM is a collection of mathematical and statistical techniques widely used to determine the effects of multiple variables and to optimize different biotechnological process (Myers and Montgomery 2002).

Catfish is a common farm-raised, warm-water fish, supplying large quantity of fish skin and bone annually. *Pangasius sutchi*, a species of catfish and known locally as “patin”, is one of the most popular freshwater fish sources in Malaysia. The total amount of pangasius catfish production in year 2007 was 5,784,444 metric tons (Department of Fisheries Malaysia 2007). Large amounts of by-product materials including bone are disposed from *P. sutchi* processing industry, which are sources of excellent high quality protein. Pangasius catfish bone, comprising about 8 % of the whole fish, has become an interesting raw material for gelatin production.

The aim of this study was to determine the optimal conditions for extraction of gelatin from *P. sutchi* bone using RSM. The extracted gelatin was characterized and its physical properties were compared with bovine and other fish species gelatin.

Materials and methods

Raw material and preparation

Pangasius catfish (*Pangasius sutchi*), 1.2 to 1.5 kg, was obtained from a farm fish located in Penang, Malaysia. The raw materials were transported to the laboratory under ice. Upon arrival, the bones were mechanically separated from fresh fish and were cleaned by scraping with a knife to eliminate some of the flesh and then degreased by tumbling in warm (35 °C) water. After tumbling, the samples were minced in a meat grinder (Model TBS 200, Taiwan), using a mesh diameter of $\varnothing=3$ mm and then the fish bones were cleaned by tap water and drained off using cheese cloth. Finally, the samples were frozen at -20 °C until use for no longer than 2 months. Commercial gelatin from bovine skin was bought from Sigma Aldrich (St. 132 Louis, MO, USA). All the chemicals used were of analytical grade.

Gelatin extraction

The degreased bone (30 g) was demineralized using HCl (1:8 w/v), at 4 °C with varying concentrations (factor X_1 , %) for varying times (factor X_2 , h), depending on the design. The leached bone (ossein) was drained off and rinsed with tap water and then neutralized using NaOH. The neutralized ossein was transferred to a beaker and extraction was carried out in distilled water (the ratio of sample/water, 1:8 w/v) at varying temperatures (factor X_3 , °C) for varying times (factor X_4 , h). The extracted solution was centrifuged for 20 min at $10,000 \times g$ at 4 °C (Thermo Scientific, High Speed Centrifuge, Sorvall HS23, Germany) and then the upper phase was filtered with a filter paper. The extracted gelatin was then concentrated with a rotary vacuum evaporator (Buchi, R-144, Germany) and dried in a freeze-dryer.

Experimental design

To optimize gelatin extraction from pangasius catfish bone, Response Surface Methodology (RSM) with a 4-factor, 5-level Central Composite Design (CCD) was adopted with hydroxyproline recovery as a response. The experimental design for the CCD step consisting of 2^4 factorial points, 8 axial points ($\alpha=2$, α indicates the number of axial point levels), and 6 replicates of the central point. After the conditions for desired range for the independent variables were set up, RSM software would provide many groups of conditions during the optimization. The ranges of four independent variables based on the results of preliminary experiments and the combination of the independent variables are shown in Tables 1 and 2.

Determination of extraction yield

Hydroxyproline has been used as an indicator to determine collagen and gelatin amount. Therefore, hydroxyproline recovery as extraction yield (Y, %) was selected for the dependent variable and calculated by the method described in AOAC 2005, with modifications. The samples were first hydrolyzed with 6 N HCl at 110 °C for 16–18 h. The hydrolyzed sample solution was filtered through Whatman no. 4 filter paper. The filtrate was neutralized with 2 N and 0.1 N NaOH to pH 6. The neutralized sample was diluted and transferred into a test tube. A 2 ml of oxidant solution containing 1.4 % (w/v) chloroamine-T, 1-propanol, at the ratio of 1:10 (v/v) and acetate/citrate buffer, pH 6, at a ratio of 1:8 (v/v)) was mixed with the diluted sample and allowed to stand for 20 min at room temperature. After that, a 2 ml of colorimetric reagent solution (a mixture of 10 % of p-dimethylaminobenzaldehyde in 35 ml of 60 % (v/v) perchloric acid (w/v) and 65 ml of 2-propanol) was added. The mixture was mixed and heated at 60 °C for 15 min and then cooled for 2–3 min in running water. Absorbance of the sample was measured against blank at 558 nm using a spectrophotometer (UV-16001,

Table 1 Independent variables and their levels in the Central Composite Design for production of pangasius catfish bone gelatin

Independent variable	Symbol	Level				
		−2	−1	0	1	2
HCl concentration (%)	X ₁	1	2	3	4	5
treatment time (h)	X ₂	0	8	16	24	32
Extraction temperature (°C)	X ₃	30	45	60	75	90
Extraction time (h)	X ₄	2	3.5	5	6.5	8

Table 2 Predicted and experimental results of the Central Composite Design for gelatin extraction from pangasius catfish bone

Standard order	Independent variable				Y	
	X ₁	X ₂	X ₃	X ₄	(Exp)	(Pred)
1	−1	−1	−1	−1	38.33	35.10
2	1	−1	−1	−1	32.65	35.07
3	−1	1	−1	−1	36.08	35.70
4	1	1	−1	−1	35.38	33.84
5	−1	−1	1	−1	60.39	57.01
6	1	−1	1	−1	62.35	56.98
7	−1	1	1	−1	60.89	60.19
8	1	1	1	−1	59.82	58.34
9	−1	−1	−1	1	40.24	41.67
10	1	−1	−1	1	47.57	47.34
11	−1	1	−1	1	38.05	42.49
12	1	1	−1	1	43.01	46.34
13	−1	−1	1	1	60.41	61.03
14	1	−1	1	1	66.39	66.72
15	−1	1	1	1	66.91	64.44
16	1	1	1	1	66.00	68.30
17	−2	0	0	0	51.83	53.17
18	2	0	0	0	57.35	56.99
19	0	0	0	0	41.47	44.69
20	0	2	0	0	49.11	46.87
21	0	0	−2	0	31.68	28.08
22	0	0	2	0	65.36	71.94
23	0	0	0	−2	37.15	43.50
24	0	0	0	2	65.39	60.03
25	0	0	0	0	57.94	61.38
26	0	0	0	0	63.37	61.38
27	0	0	0	0	63.82	61.38
28	0	0	0	0	63.41	61.38
29	0	0	0	0	57.33	61.38
30	0	0	0	0	62.39	61.38

Exp (experimental value), Pred (predicted value), X₁ (concentration of HCl, %), X₂ (treatment time, h), X₃ (extraction temperature, °C), X₄ (extraction time, h) and Y (hydroxyproline recovery, %)

Shimadzu, Kyoto, Japan). Hydroxyproline standard solutions, with concentrations ranging from 1 to 6 ppm were also run simultaneously. The blank was prepared in the same manner by using distilled water.

Proximate composition

The moisture (oven-drying procedure), crude protein (Kjeldahl method), ash and fat content (Soxhlet extraction) of the raw fish bone and extracted gelatin were estimated by the AOAC official method (AOAC 2005). The analyses were replicated three times.

Determination of gelatin yield

The optimized gelatin yield was calculated as the ratio of weight of dried gelatin to the total weight of fish ossein on wet basis using the following formula:

Yield of gelatin(%)

$$= (\text{Dry weight gelatin [g]} / \text{Wet weight of ossein [g]}) \times 100.$$

Determination of gel strength

The Bloom gel strength was determined by the British Standard 757: 1975 method (BSI 1975). A solution containing 6.67 % (w/v) gelatin was prepared in a standard Bloom jar. The mixture was later heated at 60 °C for 30 min to completely dissolve gelatin and the obtained gelatin solution was then kept in a refrigerator at 9–10 °C for 16–18 h. The gel strength was determined by TA.XT Texture Analyser (Stable Micro System, UK) equipped with a load cell of 5 kg, cross-head speed 1 mm/s and equipped with a 0.5 in. in diameter, flat bottomed plunger. The standard glass Bloom jar was placed centrally under the plunger and the penetration test was then performed. The maximum force (g) was determined when the probe proceeded to penetrate into the gel to a depth of 4 mm. The measurements were performed in triplicate.

Determination of texture profile analysis (TPA)

The gelatin samples for texture profile analysis (TPA) were prepared like those used for gel strength. After being matured at 9–10 °C for 16–18 h, the samples were equilibrated to room temperature (15 °C) for 30 min. The sample was removed from the glass bottle and TPA test was performed with the TAXT2 Texture Analyzer (Stable Micro System, UK). The gel was compressed using a 100 mm diameter aluminum plate until the deformation reached 30 % at a speed of 1.0 mm/s. The hardness, cohesiveness, springiness, gumminess and chewiness were determined as described by Pye (1996).

Determination of viscosity

The viscosity of 6.67 % (w/v) gelatin solution sample at 60 °C was analyzed by Rheometer Physica MCR 301 (Model Anton Paar, Austria) attached with 5 cm cone plate geometry with cone angle 2° and a gap set at 0.05 mm. Flow curve for each sample was obtained by shearing the sample at an increasing shear rate up to 1,400 s⁻¹ within 240 s.

Amino acid composition

Amino acid composition was performed using a high performance liquid chromatography (HPLC), equipped with a

Waters 410 Scanning Fluorescence and AccQ Tag column (3.9×150 mm). AccQ Tag Eluent A and AccQ Tag Eluent B or 60 % acetonitrile acid were used as the mobile phase (flow rate = 1 ml/min). For acid hydrolysis, 0.1 g of each sample was hydrolysed with 5 mL of 6 mol/L hydrochloric acid (HCl) in a closed test tube, and then kept in oven for 24 h at 110 °C. Samples for cysteine analysis were oxidized with performic acid before hydrolysis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The extracted gelatin was prepared for sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE) analysis according to the method described by Ghassem et al. (2011) with some modification. SDS-PAGE was conducted using the discontinuous Tris/HCl/glycine buffer system (Laemmli 1970). Gelatin extract was diluted with sample buffer containing 0.5 M Tris–HCl, pH 6.8 containing 5 % (w/v) SDS, 20 % (v/v) glycerol and 10 % (v/v) β-mercaptoethanol at the ratio of 1:1 (v/v) using 4 % stacking gel and 10 % resolving gel. The sample was run in a Mini Protein II unit (Bio-Rad Laboratories) at a constant voltage of 100 V. The gel was stained with Biosafe Coomassie G250 Stain. The load volume was 20 µL in all lines. A BIO-RAD SDS-PAGE molecular weight standard, board range was used to identify the protein fractions.

Statistical analysis

Experimental data were statistically analyzed by Design-Expert 6.0.11, (State-Ease, Inc., Minneapolis MN, USA). According to the experimental design and the response value, a 2nd order polynomial equation was chosen to represent the experimental data. As four parameters were varied, 15 β-coefficients had to be estimated which included the four main effects (linear), four quadratic effects, one constant and six interactions. Regression analysis was used for investigation. The following 2nd order polynomial equation could be considered:

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

Where Y is the dependent variable, β₀ is a constant, β_i, β_{ii}, β_{ij} are regression coefficients and X_i, X_j are levels of independent variables (i=1–4; and j=1–4).

The quadratic model is almost always sufficient for industrial applications. Therefore, it is focused on design that is useful for fitting quadratic model. This design often provides lack of fit detection that will help determine when a higher-order model is needed. The lack of fit indicates whether the calculated response surface represents the true

surface. The three-dimensional graphs were developed using Design-Expert 6.0.11, (State-Ease, Inc., Minneapolis MN, USA) and represented a function of two independent variables while keeping the other two independent variables at the optimal conditions.

After the experiments were performed, the data collected were evaluated using ANOVA and for pair comparison, t-statistic was analyzed by SPSS statistical program (Version 16.0) (SPSS Inc., Chicago, IL, USA).

Results and discussion

Optimization of gelatin processing

Development of response surface model and data analysis

A multiple regressions analysis technique was performed to determine all the coefficients of linear (X_1 , X_2 , X_3 , X_4), quadratic (X_1^2 , X_2^2 , X_3^2 , X_4^2) and interaction (X_1X_2 , X_1X_3 , X_1X_4 , X_2X_3 , X_3X_4) terms to fit a full response surface model for the response. The regression coefficients for the 2nd order response surface model in terms of coded units are shown in Table 3. In order to estimate the significance of the quadratic polynomial model equation, the analysis of variance (ANOVA) was employed (Table 4). Any term in the model, which has a small P-value and a large F-value would imply more significant effect on the relevant response variable. The P-value of 0.0001 implies that the model is significant. As a result, ANOVA proved that the predicted 2nd order model was statistically appropriate. Nevertheless, the lack of fit model was not significant ($P>0.05$) which is an

indication that the optimum model is achieved for this experiment. Moreover, the values of adjusted R-squared, predicted R-squared, R-squared, and adequate precision of this model were 0.8781, 0.6805, 0.9369 and 14.614, respectively. The high value for R-squared implies that the model can explain the high percentage of variability in the observed data. The “Predicted R-squared” and the “Adjusted R-squared” are in reasonable agreement. “Adequate Precision” value reflects the signal to noise ratio. A value greater than 4 is desirable for this ratio. The ratio in this study was 14.614, which indicates an adequate signal and implies that the model is suitable to navigate the design space. To develop the fitted response surface model equation, all insignificant terms ($P>0.05$) were eliminated and the fitted model obtained by RSM is as follows:

$$Y = -93.51 + 2.3X_3 + 12.2X_4 - 0.06X_2X_2 - 0.01X_3X_3 - 1.07X_4X_4$$

Where Y is dependent variable, and X_1 , X_2 , X_3 and X_4 are independent variables.

The changes of extraction yield affected by the independent variables were visualized through three-dimensional views of response surface plots and respective contour plots (Fig. 1a and b). The plots are represented as a function of two independent variables while keeping the other two factors at the optimal conditions. Figure 1a shows that the hydroxyproline recovery increased rapidly to an optimum value by rising in concentration of HCl and treatment time. However, further increase caused a gradual decline in the hydroxyproline recovery. As indicated in Fig. 1b, the dependent variable rose by increasing in the extraction time and temperature. Moreover, Fig. 1b illustrates how the hydroxyproline recovery increased sharply as the extraction temperature prolonged and this factor had higher impact on the dependent variable.

Conditions for optimum response

The effect of concentration of HCl (X_1), treatment time (X_2), extraction temperature (X_3) and extraction time (X_4) on the hydroxyproline recovery (Y) was determined using response surface methodology (RSM). Desirability profile was employed to establish the optimum value of each condition. Concentration of HCl (X_1), treatment time (X_2), extraction temperature (X_3) and extraction time (X_4) were set in arranged ranges, while dependent variable was fixed at maximum.

The resulting solution by response optimizer were concentration of HCl (2.74 %), treatment time (20.15 h), extraction temperature (74.73 °C) and extraction time (5.26 h). The predicted value of response was 68.75 % obtained from calculation by using the model equation with desirability of

Table 3 Regression coefficients for 2nd order response surface model in terms of coded units

	Parameter	Term	Coefficient
	β_0	Intercept	−93.51
	β_1	X_1	6.54
	β_2	X_2	1.84
	β_3	X_3	2.3
	β_4	X_4	12.2
	β_{11}	X_1^2	−1.57
	β_{12}	X_1X_2	−0.06
	β_{13}	X_1X_3	0.0002
	β_{14}	X_1X_4	0.95
	β_{22}	X_2^2	10.06
	β_{23}	X_2X_3	0.01
	β_{24}	X_2X_4	0.005
	β_{33}	X_3^2	−0.01
	β_{34}	X_3X_4	−0.03
	β_{44}	X_4^2	−1.07

X_1 (concentration of HCl, %), X_2 (treatment time, h), X_3 (extraction temperature, °C) and X_4 (extraction time, h)

Table 4 Analysis of variance (ANOVA) for response of the dependent variable (Y, %)

	Source	Sum of squares	DF	Mean square	F Value	P Value	
	Model	4016.05	14	286.86	15.9185	< 0.0001	significant
	X ₁	21.9507	1	21.9507	1.21809	0.2871	
	X ₂	7.13503	1	7.13503	0.39594	0.5387	
	X ₃	2886.62	1	2886.62	160.185	< 0.0001	significant
	X ₄	409.869	1	409.869	22.7445	0.0002	significant
	X ₁ ²	67.9285	1	67.9285	3.76949	0.0712	
	X ₂ ²	416.974	1	416.974	23.1387	0.0002	significant
	X ₃ ²	221.475	1	221.475	12.2901	0.0032	significant
	X ₄ ²	158.4	1	158.4	8.78993	0.0096	significant
	X ₁ X ₂	3.33619	1	3.33619	0.18513	0.6731	
	X ₁ X ₃	0.00013	1	0.00013	7.1E-06	0.9979	
	X ₁ X ₄	32.5758	1	32.5758	1.8077	0.1988	
	X ₂ X ₃	6.69554	1	6.69554	0.37155	0.5513	
	X ₂ X ₄	0.05218	1	0.05218	0.0029	0.9578	
	X ₃ X ₄	6.46469	1	6.46469	0.35874	0.5581	
	Residual	270.309	15	18.0206			
X ₁ (concentration of HCl, %),	Lack of Fit	226.939	10	22.6939	2.61627	0.1500	not significant
X ₂ (treatment time, h), X ₃ (ex-	Pure error	43.3706	5	8.67412			
traction temperature, °C), X ₄	Cor total	4286.36	29				
(extraction time, h)							

0.998. Verification experiment was conducted under optimal conditions to compare predicted value and actual value of dependent variable. The actual value repeated three times was 67.64 %, which was agreed well with the predicted value, indicating that both actual and predicted results confirm each other and the model is reasonable.

Proximate compositions of fish bone and gelatin

The proximate compositions of pangasius catfish bone and its gelatin are shown in Table 5. The results indicated that bone had ash content of 40.91 g/100 g and protein content of 25.65 g/100 g while its gelatin had ash and protein contents of 2.5 and 87.29 g/100 g, respectively. The protein content of the collagenous material represents the maximum possible yield of gelatin expected from them (Muyonga et al. 2004).

According to Jones (1977), lower ash content contributes to a high quality of gelatin and gelatin with a maximum ash content of 2.6 % is normally accepted for food applications. The ash content of pangasius catfish bone gelatin (2.55 g/100 g) was lower than the recommended maximum of 2.6 %. Muyonga et al. (2004) reported the ash content of Nile perch bones gelatin in rang of 7.5 to 11.2 g/100 g.

Physical properties of extracted gelatin

After optimization, the relative gelatin yield from pangasius catfish bone was 13.86 % (w/w). This yield was much

higher than those reported by Muyonga et al. (2004), for Nile perch bone gelatin (2.4 %) and Liu et al. (2009), for Channel catfish head bone gelatin (8.43 %). In addition, Rafieian et al. (2011) reported 10.2 % gelatin yield for chicken deboner residue after optimization.

The most important physical properties of gelatin are gel strength and viscosity. Commercially, gelatin with high viscosity and gel strength are preferred. The physical properties of catfish bone gelatin and comparisons with that of bovine are presented in Table 6. The gel strength of pangasius catfish bone gelatin (254 g) was comparable with bovine and much greater than other fish gelatin such as Lizardfish skin and bone (159 g and 135 g), Nile perch skin and bone (240 g and 225 g), Channel catfish head bone (209 g) and King weakfish bone (200 g) that reported by Taheri et al. (2009), Muyonga et al. (2004), Liu et al. (2009) and Alfaro et al. (2009), respectively.

Although the gel strength is one of the most important commercial criteria for gelatin, this parameter may not represent all the textural properties (Zhou and Regenstein 2007). Texture profile analysis (TPA) provides more information regarding physical characteristics of gelatin than gel strength and measures parameters such as hardness, springiness, cohesiveness, gumminess and chewiness from the TPA curve shown in Fig. 2. Table 6 shows the TPA values for these parameters of the pangasius catfish bone and bovine skin gelatin. The results show that hardness, gumminess, and chewiness of pangasius catfish bone gelatin was significantly ($P \leq 0.05$) lower than bovine gelatin but

Fig. 1 **a** and **b**. Response Surface Plots for optimization of gelatin extraction from pangasius catfish (*Pangasius sutchi*) bone. X_1 (concentration of HCl, 2.74 %), X_2 (treatment time, 20.15 h) X_3 (extraction temperature, 74.73 °C), X_4 (extraction time, 5.26 h) and Y (hydroxyproline recovery, 68.75 %)

Table 5 Proximate composition of pangasius catfish bone and its gelatin

Composition (g/100 g)	Bone	Gelatin
Moisture	24.7±0.44	9.2±0.15
Lipids	8.7±0.17	0.96±0.032
Protein	25.7±0.28	87.3±0.21
Ash	40.9±0.27	2.6±0.09

insignificant ($P>0.05$) differences were observed in springiness and cohesiveness between the two gels (Table 6).

The second most important commercial physical property of a gelatin is viscosity. The viscosity of gelatin plays a crucial role in certain food systems and high viscosities are required to stabilize food, pharmaceutical and photographic emulsions (Schrieber and Gareis 2007). Table 6 compares also the viscosity of pangasius catfish bone gelatin with bovine gelatin at 60 °C. The viscosity of gelatin extracted from

Table 6 Physical properties of gelatin from pangasius catfish bone and bovine skin

Physical properties	Fish bone gelatin	Bovine skin gelatin
Gel strength (g)	254.7±8.65 ^b	350±9.52 ^a
Viscosity (mPa s)	3.1±0.44 ^a	3.9±0.11 ^a
TPA		
Hardness (g)	242.7±9.73 ^b	341.3±34.99 ^a
Springiness	0.95±0.434 ^a	0.98±0.051 ^a
Cohesiveness (g)	0.92±0.267 ^a	0.92±0.003 ^a
Gumminess (g)	273±26.05 ^b	314±33.44 ^a
Chewiness (g)	214.2±12.92 ^b	306.1±19.01 ^a

Average ± standard deviation from triplicate determinations. Mean values in the same row with different superscript letters are significantly different ($p \leq 0.05$)

pangasius catfish bone (3.17 mPas) was closed to commercial gelatin extracted from bovine skin (3.91 mPas). When compared to other fish gelatin products, the viscosity of the extracted gelatin was higher than those for other fish gelatin including snakehead red tilapia (1.73 mPas) and cold water fish (1.55 mPas) (See et al. 2010).

Amino acid composition

The amino acid compositions of gelatin from pangasius catfish bone and bovine skin are shown in Table 7. Both of them showed high proportion of glycine, proline, hydroxyproline and glutamic acid but low in tyrosine and histidine that are property of gelatin. The imino acid (proline and hydroxyproline) content of gelatin has a strong influence on their physicochemical properties (Gilsenan and Ross-Murphy 2000). The imino acid content of

Table 7 Amino acid composition of gelatin from pangasius catfish bone and bovine skin (g/100 g protein)

Amino acids	Pangasius catfish	Bovine
Hydroxylproline	5.97	6.49
Aspartic acid	4.62	4.97
Serine	3.08	2.87
Glutamic acid	8.16	6.19
Glycine	25.02	42.71
Histidine	0.8	1.02
Arginine	8.11	7.01
Threonine	2.45	1.43
Alanine	8.48	7.73
Proline	11.4	11.09
Tyrosine	0.53	0.40
Valine	2.19	2.08
Methionine	10.83	6.43
Lysine	3.13	3.11
Isoleucine	1.33	1.35
Leucine	2.23	2.42
Phenylalanine	1.7	1.63
Cysteine	–	–
Imino acids	17.37	17.58

pangasius catfish bone and bovine gelatin were 17.37 and 17.58 g/100 g, respectively. The bovine skin gelatin indicated higher imino acid content (17.58 g/100 g) and showed higher gel strength than pangasius catfish bone gelatin. The imino acid content of pangasius catfish bone gelatin was higher than reported for salmon (16.6 g/100 g), cod (15.4 g/100 g) and bigeye snapper

Fig. 2 Schematic texture profile analysis curves of gelatin extracted from pangasius catfish (*Pangasius sutchi*) bone and bovine skin **Fig. 3** SDS-PAGE patterns of gelatin from pangasius catfish (*Pangasius sutchi*) bone: (M) molecular weight standard and (G) gelatin

(14.43 g/100 g) (Arnesen and Gildberg 2007; Binsi et al. 2009). The amino acids like tryptophan and cysteine are normally absent in a conventional gelatin.

Molecular weight distribution

Apart from amino acid composition, the average molecular weight and the distribution of α -, β -, or γ -chains are also important in measuring the gelling properties of a gelatin (Gómez-Guillén et al. 2002). Gelatin is composed of α chains, β chains (α chains dimers), higher molecular weight polymer including γ components (α chains trimers) and some lower molecular weight fragments (Sims et al. 1997). According to Zhou and Regenstein (2005), the band at 250 kDa is the β -chain and the band at 120 kDa is the α -chain. The SDS-PAGE pattern of gelatin extracted from pangasius catfish bone illustrates in Fig. 3. The board molecular weight standard protein was used as marker for the molecular weight of the gelatin. As shown in Fig. 3, SDS-PAGE pattern of extracted gelatin presents intense bands corresponding to the α_1 and α_2 chains (~116 kDa) as well as the presence of its dimers that is β -components (~200 kDa). Furthermore, some weak bands observe below 97 kDa (Fig. 3). In the present study, gelatin extracted from pangasius catfish bone showed considerable amount of low molecular weight components, indicating more degree of collagen degradation. The higher content of compounds with a low molecular weight may be an indicative of the greater breakdown of peptide bonds during gelatin processing due to the higher extraction temperature or acid process in demineralization. It was known that the degree of cross-linking is changeable among tissues and depends on the type of collagen present, age and species (Alfaro et al. 2009).

Conclusion

This study set out to determine the optimum conditions of gelatin extraction from pangasius catfish bone and characterize some properties of the extracted gelatin. With respect to the RSM application, it was concluded that pangasius catfish bone gelatin could be extracted by hot water extraction after a pretreatment with HCl. The optimum levels of concentration of acid, treatment time, extraction temperature, and extraction time in gelatin production from pangasius catfish bone were determined as 2.74 %, 20.15 h, 74.7 °C and 5.26 h, respectively, and the predicted value of hydroxyproline recovery was 68.75 %. The extracted gelatin showed desirable physical characteristics which indicating that pangasius catfish bone is a suitable raw material for gelatin production.

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References

- Alfaro AT, Costa CS, Fonseca GG, Prentice C (2009) Effect of extraction parameters on the properties of gelatin from King weakfish (*Macrodon ancylodon*) bones. *Food Sci Tech Int* 15:553–562
- AOAC (2005) Official Methods of the Association of Official Agricultural Chemist's International, 17th edn. AOAC International, Gaithersburg
- Arnesen JA, Gildberg A (2007) Extraction and characterisation of gelatine from Atlantic salmon (*Salmo salar*) skin. *Bioresour Technol* 98:53–57
- Binsi PK, Shamasundar BA, Dileep AO, Badii F, Howell NK (2009) Rheological and functional properties of gelatin from the skin of Bigeye snapper (*Priacanthus hamrur*) fish: influence of gelatin on the gel forming ability of fish mince. *Food Hydrocoll* 23:132–145
- BSI (1975) Methods for sampling and testing gelatin (physical and chemical methods). British Standards Institution, London
- Cheow CS, Norizah MS, Kyaw ZY, Howell NK (2007) Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). *Food Chem* 101:386–391
- Department of Fisheries Malaysia (2007) Estimated aquaculture production from freshwater culture system by species. http://www.dof.gov.my/c/document_library/get_file?uuid=07ab7a5d-908b-4c15-9927-874ff729490e&groupId=10131
- Ghassem M, Siau Fern S, Said M, Mohd Ali Z, Ibrahim S, Salam Babji A (2011) Kinetic characterization of *Channa striatus* muscle sarcoplasmic and myofibrillar protein hydrolysates. *J Food Sci Technol*. doi:10.1007/s1319701105266
- Gilsenan PM, Ross-Murphy SB (2000) Rheological characterization of gelatins from mammalian and marine sources. *Food Hydrocoll* 14:191–195
- GME (2008) Gelatin Manufacturers of Europe. <http://www.gelatine.org/en/gelatine/overview/127.htm>
- Gómez-Guillén MC, Turnay J, Fernández-Díaz MD, Ulmo N, Lizarbe MA, Montero P (2002) Structural and physical properties of gelatin extracted from different marine species. *Food Hydrocoll* 16:25–34
- Gómez-Guillén MC, Pérez-Mateos M, Gómez-Estaca J, López-Caballero E, Giménez B, Montero P (2009) Fish gelatin: a renewable material for the development of active biodegradable films. *Trends Food Sci Technol* 20:3–16
- Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. *Food Hydrocoll* 25:1813–1827
- Gudmundsson M, Hafsteinsson H (1997) Gelatin from cod skins as affected by chemical treatments. *J Food Sci* 62:37–47
- Jamilah B, Harvinder KG (2002) Properties of gelatins from skins of fish black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). *Food Chem* 77:81–84
- Jones NR (1977) Uses of gelatin in edible products. In: Ward AG, Courts A (eds) *The science and technology of gelatin*. Academic, London, pp 365–394
- Karim A, Bhat R (2009) Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. *Food Hydrocoll* 23:563–576
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685
- Liu HY, Han J, Guo SD (2009) Characteristics of the gelatin extracted from Channel Catfish (*Ictalurus Punctatus*) head bones. *LWT-Food Sci Technol* 42:540–544

- Montero P, Gómez-Guillén MC (2000) Extracting conditions for megrim (*Lepidorhombus boscii*) skin collagen affect functional properties of the resulting gelatin. J Food Sci 65:434–438
- Muyonga JH, Cole CGB, Duodub KG (2004) Extraction and physicochemical characterization of Nile perch (*Lates niloticus*) skin and bone gelatin. Food Hydrocoll 18:581–592
- Myers RH, Montgomery DC (2002) Response surface methodology. John Wiley & Sons Inc, New York
- Pye J (1996) Gelatin—the scientific approach to product quality. Food Aust 48:414–416
- Rafieian F, Keramat J, Kadivar M (2011) Optimization of gelatin extraction from chicken deboner residue using RSM method. J Food Sci Technol. doi:10.1007/s1319701103557
- Rawdkuen S, Sai-Ut S, Benjakul S (2010) Properties of gelatin films from giant catfish skin and bovine bone: a comparative study. Eur Food Res Technol 231:907–916
- Schrieber R, Gareis H (2007) Gelatine handbook: theory and industrial practice. Wiley-VCH, Germany
- See SF, Hong PK, Ng KL, Wan Aida WM, Babji AS (2010) Physicochemical properties of gelatins extracted from skins of different freshwater fish species. Int Food Res J 17:809–816
- Sikorski E (2001) Chemical and functional properties of food components. Gdan'sk University of Technology, Poland
- Sims TJ, Bailey AJ, Field DS (1997) The chemical basis of molecular weight differences in gelatins. The Imaging Sci J 45:171–177
- Skierka E, Sadowska M, Karwowska A (2007) Optimization of condition for demineralization Baltic cod (*Gadus morhua*) backbone. Food Chem 105:215–218
- Taheri A, Abedian Kenari AM, Gildberg A, Behnam S (2009) Extraction and physicochemical characterization of greater lizardfish (*Saurida tumbil*) skin and bone gelatin. J Food Sci 74:160–165
- Wangtueai S, Noomhorm A (2009) Processing optimization and characterization of gelatin from lizardfish (*Saurida spp.*) scales. LWT-Food Sci Technol 42:825–834
- Yang H, Wang Y, Jiang M, Oh JH, Herring J, Zhou P (2007) 2-Step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. J Food Sci 72:188–195
- Zelechowska E, Sadowska M, Turk M (2010) Isolation and some properties of collagen from the backbone of Baltic cod (*Gadus morhua*). Food Hydrocoll 24:325–329
- Zhou P, Regenstein JM (2005) Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. J Food Sci 70:392–396
- Zhou P, Regenstein JM (2007) Comparison of water gel desserts from fish skin and pork gelatins using instrumental measurements. J Food Sci 72:196–201
- Zhou P, Mulvaney SJ, Regenstein JM (2006) Properties of Alaskan pollock skin gelatin: a comparison with Tilapia and pork skin gelatins. J Food Sci 71:313–321