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Optimization of Extraction and Physicochemical Properties of Gelatin from Pangasius Catfish (*Pangasius sutchi*) Skin

(Pengoptimuman Pengekstrakkan dan Ciri Fizikomia Gelatin daripada Kulit Ikan Keli Pangasius (*Pangasius sutchi*)

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ABSTRACT

In order to optimize the extraction of gelatin from pangasius catfish skin, a response surface method (RSM) involving a Central Composite Design (CCD) was applied. Four variables, namely NaOH concentration (0-0.3 N), acetic acid concentration (0.025-0.125 N), extraction time (2-4 h) and extraction temperature (40-80°C) were selected as independent variables for the optimization using RSM. The dependent variable was calculated by hydroxyproline recovery. The optimum conditions for extraction were produced by a pre-treatment of 0.2 N NaOH and 0.1 N acetic acid along with hot water extraction at 63.7°C for 2.41 h. The results showed that the predicted response by RSM (68.53%) closely matched the experimental response of 68.16%. The results indicated that the extracted gelatin possessed high gel strength (438 g) and high content of imino acid (proline and hydroxyproline) (18.01%) with a viscosity of 4.67 mPa s. The results showed that RSM was a great optimizing tool for extraction of gelatin from pangasius catfish skin. The gelatin was also proven to have significantly (p<0.05) higher quality of physicochemical properties than those from bovine skin gelatin.

Keywords: Gelatin; optimization; pangasius catfish; physicochemical properties; response surface method

ABSTRAK

Kaedah Permukaan Respons (RSM) yang melibatkan reka bentuk komposit tengah (CCD) telah digunakan untuk mengoptimumkan pengeluaran gelatin daripada kulit ikan keli pangasius. Empat parameter, iaitu kepekatan NaOH (0-0.3N), kepekatan asid asetik (0.025-0.125 N), masa pengekstrakan (2-4 jam) dan pengekstrakan suhu (40-80°C) telah dipilih sebagai parameter tidak bersandar untuk tujuan ini. Parameter bersandar diukur melalui pemulihan hidroksiprolin. Keadaan optimum bagi pengekstrakan diperoleh dengan pra-perlakuan NaOH 0.2 N dan 0.1 N asid asetik dan pengekstrakan dengan menggunakan air panas pada suhu 63.7°C selama 2.41 jam. Keputusan menunjukkan bahawa respons yang diramalkan oleh RSM (68.53%) menghampiri nilai uji kaji, iaitu 68.16%. Keputusan menunjukkan ekstrak gelatin mempunyai kekuatan gel (438 g) dan mengandungi asid imino yang tinggi (prolin dan hidroksiprolin) (18.01%) dengan nilai kelikatan 4.67 mPa s. Ini menunjukkan bahawa RSM adalah kaedah yang efisien digunakan untuk mengoptimumkan pengeluaran gelatin daripada kulit ikan keli pangasius. Gelatin daripada kulit ikan keli juga telah terbukti mempunyai kualiti fizik-kimia yang lebih tinggi secara signifikan (p<0.05) berbanding gelatin daripada kulit lembu.

Kata kunci: Ciri fizikokimia; gelatin; ikan keli pangasius; kaedah permukaan respons; pengoptimuman

INTRODUCTION

Gelatin is a polypeptide with a high molecular weight and an important hydrocolloid, broadly used to improve stability, elasticity and consistency in food products. It is also known as the denatured and partially hydrolyzed collagen and obtained from the skin, connective tissue and bones of animals. The worldwide production amount of gelatin is about 300,000 tons per year and its global demand has been increasing over the years (Schrieber & Gareis 2007). Due to the increasing demand for non-mammalian gelatin for halal and kosher food markets, greater interest and focus was generated on development of methods for efficient utilization of fish by-products to produce fish gelatin as replacements for mammalian sources (Karim & Bhat 2008). Gelatin manufacturing processes consist of two main stages; pre-treatment of the raw material and hot-water extraction. 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 pretreatment and the extraction processes (Gómez-Guillén et al. 2011). Pre-treatments of raw materials for gelatin extraction were divided into two distinctive categories: An alkaline process and an acid process. A combination of these pre-treatments was carried out, including an alkaline pretreatment and subsequently, acid neutralization. This method not only removed non-collagenous proteins, but also resulted in a gelatin with good gel property and high gelatin yield, probably due to a neutral or weak acid extraction medium (Zhou & Regenstein 2005).

The quality of gelatin depends on its physicochemical properties, which were greatly influenced, not only by the origin of raw material, but also by the processing methods and parameters (Cheow et al. 2007). The main problem of fish gelatins is that their gels tend to be less stable and have poorer gelling properties than gelatins 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 tropical and sub-tropical warm-water fish species (tilapia, Nile perch, catfish) might have similar physicochemical properties to that of mammal gelatins, depending on the species, type of raw material and processing conditions (Gilsenan & Ross-Murphy 2000; Gómez-Guillén et al. 2009; Jamilah & Harvinder 2002; Muyonga et al. 2004).

Research on fish gelatin is important for the development of methods to produce gelatins in large scale and good quality (Montero & 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. 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 & Montgomery 2002).

In relation to this study, catfish is used as a subject as it is a common farm-raised, warm-water fish, supplying large quantity of fish skins annually in Asia. Pangasius catfish (P. sutchi), known locally as 'patin', is one of the most popular freshwater fish sources in Malaysia. According to the Department of Fisheries Malaysia (2007), the total amount of pangasius catfish production in year 2007 was 5,784,444 metric tons. Pangasius catfish skin, comprising about 6% of the whole fish, has become an interesting raw material for gelatin production.

The aim of this study was to determine the optimal condition for gelatin extraction from pangasius catfish skin using RSM. The extracted gelatin was characterized and its physicochemical properties were compared with bovine and other fish species gelatin.

Materials and Methods

MATERIALS PREPARATION

Pangasius catfish (*P. sutchi*) weighing around 1.2 to 1.5 kg were obtained from a farm fish located in Penang, Malaysia. The raw materials were transported to the laboratory under ice. Upon arrival, the fishes were filleted and the skin was manually removed by using a sharp knife. After filleting, the fish skins were cleaned by tap water for three times and drained. The fish skins were then cut into 2 × 2 cm pieces and frozen at -20ºC with a maximum storage of less than 2 months before use. Commercial gelatin from bovine skin was bought from Sigma Aldrich (St. Louis, MO, USA). All the chemicals used were of analytical grade.

GELATIN EXTRACTION

The cleaned skins (30 g) were treated with NaOH (1:8 w/v) with varying OH^- concentrations (factor X_1, N) for 60 min. The samples were then drained and rinsed 3 times with tap water. Samples were then treated with varying concentrations of acetic acid (1:8 w/v, factor X_2, N) for 60 min. The samples were then drained and rinsed 3 times with tap water. The samples were subsequently mixed with distilled water (the ratio of sample/water, 1/8 w/v) and extracted at varying temperatures (factor X_3, °C) for varying times (factor X_4, min). Based on the preliminary experiments, other factors were given a fixed value for all the experiments performed thereafter as follows: Pretreatment time at 60 min, pretreatment temperature at 4°C and the skin/water ratio at 1:8 (w/v). The extracted gelatin was then concentrated with a rotary vacuum evaporator (Buchi, R-144, Germany) before drying in a freeze-dryer.

EXPERIMENTAL DETAILS

In order to optimize gelatin extraction from pangasius catfish skin, response surface methodology (RSM) with 4-factors, 5-levels central composite design (CCD) was adopted. Hydroxyproline content has been used as indicator to determine collagen and/or 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 slight modification.

The experimental design for the CCD step consisting of 2^4 factorial points, 8 axial points (α =2, α indicates the number of axial point levels) and 6 replicates of the central point. After the conditions for the desired range for the independent variables were set up, the RSM software would provide many groups of optimized conditions. The ranges of four independent variables based on the results of preliminary experiments and the combination of the independent variables were shown in Tables 1 and 2.

STATISTICAL ANALYSIS

The 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 second-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 second-order polynomial equation could be considered:

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

TABLE 1. Independent variables and their levels in the central composite design for production of pangasius catfish (*P. sutchi*) skin gelatin

Independent variable	Symbol	Level				
		–2	–1	0	1	2
NaOH concentration (N)	X1	0	0.075	0.15	0.225	0.3
Acetic acid concentration (N)	X2	0.025	0.05	0.075	0.1	0.125
Extraction temperature (˚C)	X3	40	50	60	70	80
Extraction time (h)	X4	2	2.5	3	3.5	4

TABLE 2. Predictive and experimental results of the central composite design for gelatin extraction from pangasius catfish (*P. sutchi*) skin

Standard order	Independent variable					
	x_1	x_2	x_3	x_4	Y (Exp)	Y (Pred)
1	–1	–1	–1	–1	52.5345	47.61812
2	1	–1	–1	–1	47.9833	50.95947
3	–1	1	–1	–1	56.1504	55.64895
4	1	1	–1	–1	64.3774	60.51987
5	–1	–1	1	–1	54.4326	58.49618
6	1	–1	1	–1	67.2577	64.22145
7	–1	1	1	–1	61.4802	59.28214
8	1	1	1	–1	65.2553	66.53698
9	–1	–1	–1	1	57.766	56.60435
10	1	–1	–1	1	56.811	54.84112
11	–1	1	–1	1	62.6776	61.5459
12	1	1	–1	1	65.2558	61.31225
13	–1	–1	1	1	65.2275	64.91709
14	1	–1	1	1	64.9163	65.53778
15	–1	1	1	1	65.4699	62.61377
16	1	1	1	1	64.0156	64.76404
17	–2	0	0	0	56.0427	58.52485
18	2	0	0	0	62.4507	64.01646
19	0	–2	0	0	58.5385	58.38121
20	0	2	0	0	61.4331	65.6383
21	0	0	–2	0	37.6587	42.88773
22	0	0	2	0	58.3987	57.21758
23	0	0	0	–2	60.845	61.91516
24	0	0	0	2	66.1507	69.12845
25	0	0	0	0	62.5884	65.79935
26	0	0	0	0	67.0104	65.79935
27	0	0	0	0	66.5774	65.79935
28	0	0	0	0	67.7835	65.79935
29	0	0	0	0	68.537	65.79935
30	0	0	0	0	62.2994	65.79935

X_1 (Alkaline concentration, N), X_2 (Acid concentration, N), X_3 (Extraction temperature, ˚C), X_4 (Extraction time, h) and Y (Extraction yield, %)

where Y is the dependent variable, β_0 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.

For the models calculated, analysis of variance (ANOVA) was performed and for pair comparison, t-statistic was analyzed by SPSS statistical program (Version 16.0) (SPSS Inc., Chicago, IL, USA).

PROXIMATE COMPOSITION

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

GELATIN YIELD

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

> Yield of gelatin (%) = (weight of freeze dried gelatin [g]/wet weight of fresh skin [g]) × 100.

GEL STRENGTH

The gel strength was determined by the British Standard 757: 1975 method (BSI 1975). A 6.67% (w/v) gelatin solution was prepared by mixing 7.5 g of the extracted gelatin and 105 mL of distilled water. The mixture was left at room temperature for 30 min until completely dispersed. The Bloom bottles were then transferred to a water bath maintained at a temperature below 60°C for 20 min to completely dissolve gelatin. The samples were then transferred to a cold water bath maintained at 10±0.1°C and held at this temperature for 16-18 h before determination of gel strength. The gel strength was determined by using the TAXT2 texture analyzer (Stable Micro System, UK) equipped with a load cell of 5 kg, cross-head speed 1 mm/s and equipped with a 0.5 inch in diameter, flat bottomed plunger and the measurements were performed in triplicate.

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 samples were removed from the glass bottles and the TPA test were 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).

VISCOSITY

The viscosity of 6.67% (w/v) gelatin solution samples at 60°C were 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 curves for each sample were obtained by shearing the samples at an increasing shear rate up to 1400 s^{-1} within 240 s.

AMINO ACID COMPOSITION

Amino acid composition was performed using a high performance liquid chromatography (HPLC). 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. The samples for cysteine analysis were oxidized with performic acid before hydrolysis.

RESULTS AND DISCUSSION

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 responses. The quadratic model in this experiment was appropriate to the response of Y with high R-squared value 0.9354. The high value for R-squared implies that the model can explain the high percentage of variability in the observed data.

In order to estimate the significance of the quadratic polynomial model equation, the analysis of variance (ANOVA) was employed (Table 3). Any terms 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. Overall, the analysis of variance suggested that the predicted quadratic model for gelatin extraction conditions of pangasius catfish skin was statistically valid. The final response surface regression equation obtained by RSM is as follows:

$$Y = -185.21 + 161.69\, X_1 + 594.40\, X_2 + 5.9\, X_3 + 0.25\, X_4 - 338.31\, X_1X_1 - 749.17\, X_2X_2 - 0.04\, X_3X_3 - 0.0002\, X_4X_4 - 129.39\, X_1X_2 + 0.79\, X_1X_3 - 0.4\, X_1X_4 - 3.74\, X_2X_3 - 1.03\, X_2X_4 - 0.00005\, X_3X_4,$$

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

Figure 1(a) and 1(b) illustrates the changes of dependent variable affected in terms of the independent variables through a three-dimensional view of respective contour plot and response surface plot. The plots were drawn in terms of a function of two independent variables while the other two variables were kept at their optimal conditions. Figure 1(a) shows that the extraction yield increased sharply with the increase of temperature up to 65°C, after which the yield of extraction is declined. Figure 1(a) also indicates that the extraction yield is increased gradually by the increase in the concentration of NaOH.

TABLE 3. Analysis of variance (ANOVA) for response of the dependent variable (Y, %)

Source	Sum of squares	DF	Mean square	F Value	*p* Value
Model	1142.87	14	81.63	15.51	< 0.0001
X_1	91.84	1	91.84	17.44	0.0008
X_2	41.46	1	41.46	7.87	0.0133
X_3	352.51	1	352.51	66.95	<0.0001
X_4	12.44	1	12.44	2.36	0.1451
X_1^2	99.33	1	99.33	18.87	0.0006
X_2^2	6.01	1	6.01	1.14	0.3021
X_3^2	544.99	1	544.99	103.51	<0.0001
X_4^2	1.27	1	1.27	0.24	0.6304
X_1X_2	0.94	1	0.94	0.18	0.6783
X_1X_3	5.68	1	5.68	1.08	0.3153
X_1X_4	12.99	1	12.99	2.47	0.1370
X_2X_3	14.02	1	14.02	2.66	0.1235
X_2X_4	9.54	1	9.54	1.81	0.1982
X_3X_4	0.00	1	0.00	0.00	0.9777
Residual	78.97	15	5.26		
Lack of fit	61.84	10	6.18	1.80	0.2670
Pure error	17.13	5	3.43		
Cor total	1221.84	29			

X_1 (Alkaline concentration, N), X_2 (Acid concentration, N), X_3 (Extraction temperature, °C), X_4 (Extraction time, h) and Y (Extraction yield, %)

Figure 1(b) illustrates how the extraction yield is raised gradually as the extraction time is prolonged. Figure 1 also shows that the extraction temperature factor had higher impact on the extraction yield.

CONDITIONS FOR OPTIMUM RESPONSE

For the optimization of gelatin extraction, four independent variables were selected, which included a concentration of 0.2 N NaOH, a concentration of 0.1 N acetic acid, an extraction temperature of 63.70°C and an extraction time of 2.41 h. The actual experimental yield of gelatin (68.16%) agreed well with the predicted value (68.53%) with a desirability of 1.000 obtained by the RSM.

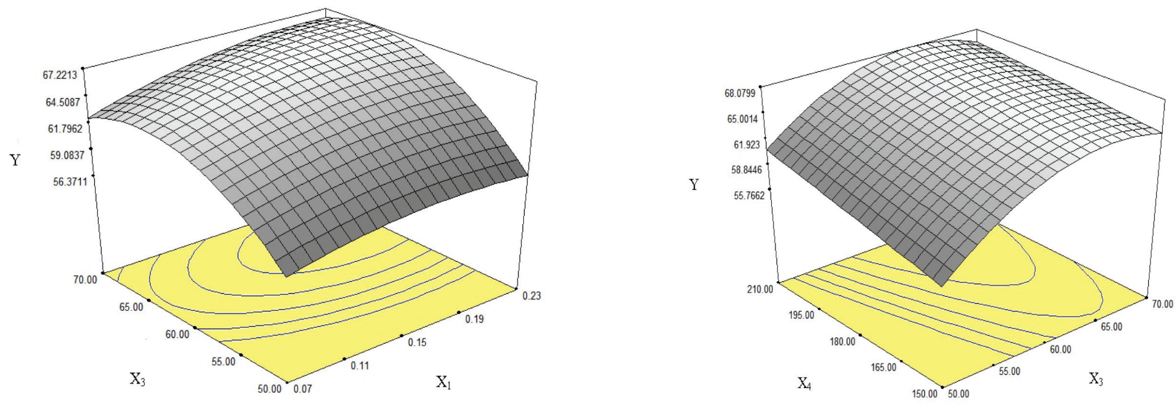
PROXIMATE COMPOSITION

The proximate compositions of pangasius catfish skin and its gelatin are shown in Table 4. The results indicated that skin had protein content of 30.91% while its gelatin had protein contents of 91.33%. Muyonga et al. (2004) explained that the protein content of the collagenous material represents the maximum possible yield of gelatin expected from them. Lower ash content contributes to a high quality of gelatin and gelatins with a maximum ash content of 2.6% are normally accepted for food applications (Jones 1977). The ash content of pangasius catfish skin gelatin (1.38%) was lower than the recommended maximum of 2.6%.

PHYSICAL PROPERTIES OF EXTRACTED GELATIN

After optimization, the relative gelatin yield from pangasius catfish skin was 23.61% (w/w). This yield was significantly higher than those reported by See et al. (2010), for gelatin from snakehead (16.57%) and red tilapia (11.75%). Gelatin yield from pangasius catfish skin showed even much higher results when compared with gelatin from other fish species such as: black tilapia (5.4%), red tilapia (7.8%) (Jamilah & Harvinder 2002), bigeye snapper (4%) (Binsi et al. 2009), megrim (7.4%), sole (8.3%), cod (7.2%) and hake (6.5%) (Gómez-Guillén et al. 2002).

The most important physical properties of gelatin are gel strength and viscosity. Commercially, gelatin with high viscosity and gel strength are preferred and are most expensive (Zhou et al. 2006). Typically, fish gelatins possess less gel strength compared to gelatins obtained from mammalian sources (Gilsenan & Ross-Murphy 2000). However, gelatins obtained from some warm-water fish species have shown high gel strength, close to that of mammalian gelatin (Choi & Regenstein 2000). The physical properties of pangasius catfish skin gelatin and their comparison to bovine gelatin are presented in Table 5. In this study, gelatin extracted from pangasius catfish skin exhibited a significantly ($p<0.05$) higher gel strength and viscosity than that of bovine (Table 5). The gel strength of the pangasius catfish skin gelatin (438 g) was much greater than those for other fish skin gelatins including salmon



(a) (b)

FIGURE 1. (a) and b). Response surface plots for optimization of gelatin extraction from pangasius catfish (*P. sutchi*) skin. X_1 (concentration of NaOH, N), X_3 (extraction temperature, °C), X_4 (extraction time, min) and Y (extraction yield, %)

TABLE 4. Proximate composition of pangasius catfish (*P. sutchi)* skin and gelatin

Composition (g/100g)	Skin	Gelatin
Moisture	50.03 ± 0.27	7.05 ± 0.07
Lipid	6.95 ± 0.17	0.23 ± 0.13
Protein	30.91 ± 0.28	91.33 ± 0.21
Ash	4.14 ± 0.18	1.38 ± 0.15

(108 g), cod (90 g), hake (110 g), Alaska Pollock (98 g), yellowfin tuna (426 g) and Nile tilapia (240 g) (Arnesen & Gildberg 2007; Cho et al. 2005; Gómez-Guillén et al. 2002; Muyonga et al. 2004; Yang et al. 2007; Zhou et al. 2006).

The viscosity of gelatin extracted from pangasius catfish skin (4.67 mPa s) was higher than commercial gelatin extracted from bovine skin (3.17 mPa s). When compared with other fish gelatin products, the viscosity of the extracted gelatin from pangasius catfish skin was relatively higher than those of other fish gelatin including snakehead (3.82 mPa s), red tilapia (1.73 mPa s) and cold water fish (1.55 mPa s) (See et al. 2010).

Although the gel strength is one of the important commercial criteria for gelatin, this parameter may not represent all the textural properties encountered during human consumption of the product (Zhou & Regenstein 2004). 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 Figure 2. Table 5 shows the TPA values for these parameters of the pangasius catfish skin and bovine skin gelatin. The results showed that hardness, gumminess and chewiness of

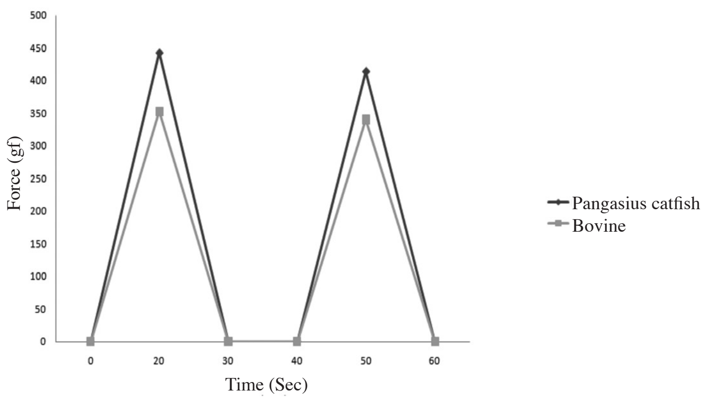


FIGURE 2. Schematic texture profile analysis curves of gelatin extracted from pangasius catfish (*P. sutchi*) and bovine skin

TABLE 5. Physical properties of gelatin pangasius catfish (*P. sutchi*) skin and bovine skin

Physical properties	Fish skin gelatin	Bovine gelatin
Gel strength (g)	438.34 ± 5.55 [a]	349.98 ± 4.52[b]
Viscosity (mpas)	4.67 ± 0.3[a]	3.17 ± 0.44[b]
TPA		
Hardness (g)	435 ± 6.10 [a]	341 ± 35.00 [b]
Springiness	0.976 ± 0.05 [a]	0.977 ± 0.06 [a]
Cohesiveness (g)	0.955 ± 0.02 [a]	0.92 ± 0.00 [a]
Gumminess (g)	415 ± 7.17 [a]	314 ± 33.44 [b]
Chewiness (g)	405 ± 23.76 [a]	306 ± 19.00 [b]

[a,b] Average ± standard deviation from triplicate determinations. Means in the same raw with different superscript letters are significantly different ($p \leq 0.05$)

pangasius catfish skin gelatin was significantly ($p<0.05$) higher than bovine gelatin but insignificant differences ($p>0.05$) were observed in springiness and cohesiveness between the two gels (Table 5).

AMINO ACID COMPOSITION

According to Gomez-Guillen et al. (2002), imino acid content (proline and hydroxyproline) is important because it affects the functional properties of gelatin. The amino acid compositions of gelatin from pangasius catfish and bovine skin are shown in Table 6. It was evident that both gelatins showed high proportion of glycine, proline, hydroxyproline and glutamic acid but low in methionine, tyrosine and histidine, which are the inherent property of all gelatins. In the present study, the pangasius catfish skin gelatin indicated higher imino acid content (18.01%) than bovine skin gelatin (17.57%) and showed higher viscosity and gelling properties than bovine gelatin. The amino acids like tryptophan and cysteine are normally absent in a conventional gelatin. However, the presence of cysteine at low concentration in gelatin from pangasius catfish skin has been observed in this study (Table 6).

CONCLUSION

This study showed that pangasius catfish skin can be successfully used in gelatin production. The optimum conditions of gelatin extraction from pangasius catfish skin was successfully determined by using RSM application. It was concluded that alkaline and acetic acid concentration as well as extraction temperature and time significantly affected the extraction process. In comparison to bovine gelatin, the gelatin extracted from pangasius catfish skin possessed significantly higher physicochemical properties.

TABLE 6. Amino acid composition of gelatin from pangasius catfish (*P. sutchi*) skin and bovine skin (g/100 g protein)

Amino acids	Pangasius catfish (%)	Bovine (%)
Hydroxylproline	6.31	6.49
Aspartic acid	5.33	4.97
Serine	3.62	2.87
Glutamic acid	7.94	6.19
Glycine	23.34	42.71
Histidine	0.75	1.02
Arginine	7.55	7.01
Threonine	3.25	1.43
Alanine	9.77	7.73
Proline	11.7	11.09
Tyrosine	0.61	0.40
Valine	2.68	2.08
Methionine	7.48	6.43
Lysine	3.51	3.11
Isoleucine	1.64	1.35
Leucine	2.74	2.42
Phenylalanine	1.85	1.63
Cysteine	0.01	-
Imino acids	18.01	17.57

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