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Optimization of gelatin extraction and physico-chemical properties of catfish (*Clarias gariepinus*) bone gelatin

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Abstract

The extraction of catfish (*Clarias gariepinus*) bone gelatin was optimized by using Response Surface Methodology (RSM) involving 4-factors, 5-levels Central Composite Design (CCD). The optimum conditions for extraction were produced by a pre-treatment of 3.35% HCl for 14.5 h along with hot water extraction at 67.23°C for 5.2 h. Results showed that the predicted yield by RSM (61.81%) was closely matched the experimental yield of 60.54%. The results also indicated that the extracted bone gelatin possessed high protein content (81.75%) and imino acid (proline and hydroxyproline) (144 residues per 1000 residues), with gel strength (230.25 g), viscosity (4.64 mPa.s) and isoionic point (5.35) comparable to that of bovine gelatin. The results suggested that RSM is a great optimizing tool for extraction of gelatin from clarias catfish bone and values of the physicochemical properties of gelatin are higher or comparable than those from other fish species and bovine gelatin.

Introduction

Gelatin is a polypeptide with high molecular weight obtained by partial hydrolysis of collagen which is found in connective tissues, bones, and some intestines of animals (Mohtar *et al*., 2010). Gelatin is a multipurpose natural product having a many applications particularly in the food industry. Most commercial sources of gelatin are from mammalian sources mostly bovine bones or porcine skins. Due to superior gel qualities (gel strength and viscosity) of gelatins from land animal sources, they are more popular compared to those from marine sources. However, the outbreak of mad cow disease or bovine spongiform encephalopathy (BSE) has opened the opportunity for marine source gelatins as an alternative. Another aspect is that fish gelatin meets the requirements of Muslims/Jews who consume Halal/Kosher gelatin as well as Hindus, who don't consume bovine products (Mohtar *et al*., 2010). Gom´ez-Guill´en *et al*. (2002) reported that 30% of the waste is in the form of bones and skins. The fish bones can be processed into gelatin, thus contribute to solve the problem of waste disposal and in addition creating a value-added product. Therefore, extraction of gelatin from fish skin or bone is of interest. There have many studies regarding the process of gelatin extraction from different kinds of fish bones or skins, such as nile perch skin and bone (Muyonga *et al*., 2004), shark cartilage (Cho *et al*., 2004), yellow fin tuna skin (Cho *et al*., 2005), cod head (Arnesen and Gildberg, 2006), grass carp fish skin (Kasankala *et al*., 2007), channel catfish skin (Liu *et al*., 2008), lizardfish skin and bone (Taheri *et al*., 2009) and hoki skin (Mohtar *et al*., 2010).

Catfish is a common farm-raised, warm-water fish, supplying large quantity of fish skins annually. The gels prepared from catfish skin are relatively thermally non-degradable and show good gelling ability (Gómez-Guillén *et al*., 2011). Until now, gelatin from the bones of catfish has not been systematically studied as a raw material for edible gelatin. According to Department of Fisheries Malaysia (2007), the total amount of catfish production in year 2007 was 21,891.55 metric tons. The sale of catfish produced in Malaysia in year 2007 earned RM 107 million out of the total aquaculture fish production of RM 481 million.

In the process of gelatin extraction, factors such as treatment concentration, treatment time and temperature, extraction time and temperature will influence the yield of gelatin. In this context, the main goal of gelatin extraction from catfish bone was to obtain the maximum possible yield of hydroxyproline

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recovery. Optimization is one of the methods to find the best alternative from a specified set of alternatives. It is the modern statistically derived experimental designs that are viewed as a way to achieve this purpose at the lowest possible overall cost (Arteaga *et al*., 1996). Response surface methodology (RSM) has been effective in the optimization and monitoring of food processes (Wangtueai and Noomhorm, 2009). It is a collection of mathematical and statistical modeling technique that relates product treatment to the outputs and establishes a regression equation to describe inter-relations between input parameters and product properties (Cho *et al*., 2004). The aim of this study were to determine optimal conditions of catfish bone gelatin extraction. Some of the physicochemical characteristics of the extracted catfish bone gelatin were also compared with those of commercial bovine gelatin.

Materials and Methods

Material and preparation

Frozen catfish (*Clarias gariepinus*) were obtained from Penang Island in Peninsular Malaysia. After filleting, this item was stored at -20ºC for maximum 2 months until further usage. The length of fishes was ranged between 40 and 50 cm. Preparation of raw material was done by filleting manually. The bones were cleaned to remove attached flesh by scraping with a knife and subsequently degreased by tumbling in warm water (35°C), before being segmented in to small pieces. Bovine skin was bought from Sigma Aldrich for comparison. All reagents used, were of analytical grade.

Gelatin extraction

The cleaned bones were treated at 4°C with different concentrations of HCl (1-5%) for varied times periods (0-32 h) to demineralize. The bones were then neutralized by washing them under tap water until their pH reached 7. The bones were washed again with distilled water to remove any tap water residuals. The fish bones were mixed with distilled water at a ratio of 1:8 (bone/water (w/v)) in a flask and gelatin was extracted at different temperatures (30-90°C) for varied times periods (2-8 h). Finally, the gelatin solution was filtered through 4 layers of cheesecloth, and subsequently centrifuged at 10,000 g at 4ºC for 20 min. Hydroxyproline content of gelatin extracted was determined according to AOAC method (2000) with modification. The extracted gelatin solutions were concentrated and then freeze dried and kept for analysis.

Proximate composition

Based on the procedures of the AOAC (2000), proximate analysis of raw catfish bone and extracted gelatin were carried out. The moisture content was determined according to oven method (AOAC, 2000). The total crude protein content was determined using Kjeldahl method (AOAC, 2000). For calculation of crude protein content of extracted gelatin and raw fish bone, a nitrogen conversion factor of 5.4 and 6.25 were used, respectively. Total lipid content of samples was evaluated by Soxhlet extraction (AOAC, 2000). Ash content was determined by charring the pre dried sample in crucible at 600°C until a white ash was formed (AOAC, 2000).

Gelatin yield

The ratio of dried gelatin weight to the total fish bone weight on wet basis was used as the gelatin yield.

$$\text{Yield of gelatin (\%)} = (\text{weight of dried gelatin [g]} / \text{wet weight of fresh bone [g]}) \times 100$$

Gel strength

Gel strength determination was done based on 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 60ºC for 30 min to dissolve gelatin completely. The gelatin solutions were cooled at room temperature for 30 min before being chilled in a refrigerator at 7ºC for 18 h. The samples were assumed to be at a temperature of 7ºC since the gel strength was measured immediately after being removed from 7ºC refrigeration using a 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 inch 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 done in triplicate.

Texture profile analysis (TPA)

TPA was measured using the Texture analyzer (TA.XT Plus). Gelatin gel samples were formed by using the same samples as used for the gel strength experiment. After gel maturation, the gels were removed from the glass bottles. The cylindrical gelatin samples were 35 mm in diameter and 25 mm in height. The samples were lubricated with mineral oil. The gels were compressed by an aluminum probe (100 mm diameter plate) until the deformation reached 30% at

Table 1. Independent variables and their levels in the 4-factors, 5-levels central composite design for optimizing the extraction condition of catfish (*Clarias gariepinus*) bone gelatin

Independent variables	Symbol	Levels				
		Code values				
		-2	-1	0	1	2
		Real values				
Concentration of HCl (%)	X_1	1	2	3	4	5
Pretreatment time (h)	X_2	0	8	16	24	32
Extraction time (h)	X_3	2	3.5	5	6.5	8
Extraction temperature (°C)	X_4	30	45	60	75	90

Table 2. Predictive and experimental results for the central composite design for gelatin extraction from catfish (*Clarias gariepinus*) bone

	Independent variable				Yield (%)	
Standard order	X_1	X_2	X_3	X_4	Experimental	Predicted
1	−1	−1	−1	−1	32.69	31.72
2	1	−1	−1	−1	33.77	32.82
3	−1	1	−1	−1	27.70	25.50
4	1	1	−1	−1	26.48	30.55
5	−1	−1	1	−1	28.39	29.64
6	1	−1	1	−1	37.48	38.10
7	−1	1	1	−1	27.02	28.90
8	1	1	1	−1	45.13	41.33
9	−1	−1	−1	1	40.13	43.40
10	1	−1	−1	1	48.29	46.88
11	−1	1	−1	1	40.33	40.19
12	1	1	−1	1	49.40	47.63
13	−1	−1	1	1	38.98	35.38
14	1	−1	1	1	44.56	46.23
15	−1	1	1	1	37.25	37.67
16	1	1	1	1	51.02	52.47
17	−2	0	0	0	25.88	25.90
18	2	0	0	0	45.39	45.41
19	0	−2	0	0	21.09	21.12
20	0	2	0	0	57.42	57.45
21	0	0	−2	0	46.43	46.46
22	0	0	2	0	33.90	33.93
23	0	0	0	−2	23.12	23.15
24	0	0	0	2	50.76	50.79
25	0	0	0	0	57.56	59.48
26	0	0	0	0	62.77	59.48
27	0	0	0	0	57.35	59.48
28	0	0	0	0	57.71	59.48
29	0	0	0	0	59.92	59.48
30	0	0	0	0	61.56	59.48

X_1: Concentration of HCl (%), X_2: pre-treatment time (h), X_3: Extraction time (h), X_4: Extraction temperature (°C) Y: yield of hydroxyproline recovery

a speed of 1.0 mm/s. The pause between the first and second compressions was 3 s. The testing was done immediately after the samples were removed from the refrigerator. Five measurements were made for each sample in the same lot. From the force-time curve of the texture profile, textural parameters including hardness, springiness, cohesiveness, gumminess and chewiness were obtained by according the method of Yang *et al*. (2007).

Viscosity

The shear viscosity was determined using a Rheometer Physica MCR 301(Model Anton Paar, Austria) with a 5 cm cone plate and a cone angle of 2º and a gap set at 0.05 mm. Using a micropipette with a tapered tip attachment, the rheometer was filled with approximately 0.5 ml of the sample solutions. By shearing the samples within 240 s at an increasing shear rate up to 1400 s^{-1}, the flow curves of each sample were obtained. During the measurements, the temperature of the samples was kept at 60ºC. Using the built in software provided with the instrument, the shear rate-stress data were fitted to a Newtonian model.

Amino acid composition

The sample of catfish bone gelatin extracted was hydrolyzed for 16 h in 15 mL of 6N HCl at 110ºC. The sample was dissolved in deionized water and filtered. The amino acid composition was obtained using a high performance liquid chromatography (HPLC), equipped with a Waters 410 Scanning Flourescence and AccQ Tag column (3.9 x 150 mm). AccQ Tag Eluent A and AccQ Tag Eluent B or 60% acetonitrile acid was used as the mobile phase (flow rate=1 ml/min).

Isoionic point

The isoionic point was determined by passing a 1.0% (w/v) solution of gelatin through a column of mixed bed resin (Amberlite IR 120 & IRA 400, Rohm and Hass Co.) until constant pH of deionised solution was obtained.

Optimization experimental design

To optimize gelatin extraction from catfish bones, Response Surface Methodology (RSM) was adopted in this study. RSM is a collection of mathematical techniques and statistical applications for formulating, improving and optimizing processes (Myers and Montgomery, 1995). A 4-factors, 5-levels Central Composite Design (CCD) was used to optimize the experiments. The central composite design was composed of 30 treatments including 24 factorial points, six replicates of the central point and eight axial points. The four independent variables were concentration of HCl (X_1, %), treatment time (X_2, h), extraction time (X_3, h) and extraction temperature (X_4, °C) were coded to five different ranges of -2,-1, 0, +1, +2 (Table 1). The design of experiments and dependent variable values are presented in Table 2. Yield of hydroxyproline recovery was selected as dependent variables. RSM was employed to better understand the interactive effects of the independent variables on the dependent variable (Y, %) to generate optimum conditions to achieve maximum yield in gelatin extraction (Mohtar *et al*., 2010).

Statistical analysis

The response surface methodology (RSM) was statistically analyzed by Design-Expert, Version 6.0.11 software (Stat-ease Inc., Minneapolis, Minn.,

U.S.A.). The multiple regressions analysis was performed by the taking into account the main, quadratic and interaction effects to develop a quadratic polynomial equation. As four parameters were varied, 19 β-coefficients had to be estimated which included coefficients for the four main effects, four quadratic effects, six interactions, four cubic and one constant. It is assumed that the estimated behavioral model of dependent variable was described by a third degree polynomial equation:

$$Y= \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_1^1 X_1^2 + \beta_2^2 X_2^2 + \beta_3^3 X_3^2 + \beta_4^4 X_4^2 + \beta_1^2 X_1 X_2 + \beta_1^3 X_1 X_3 + \beta_1^4 X_1 X^4 + \beta_2^3 X_2 X^3 + \beta_2^4 X_2 X_4 + \beta_3^4 X_3 X_4 + \beta_1^1 X_1^3 + \beta_2^2 X_2^3 + \beta_3^3 X_3^3 + \beta_4^4 X_4^4$$

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 R^2 value and the lack of fit value were determined. After the multifactor analysis of variance and the third degree model prediction determinations, the optimal gelatin extraction conditions were obtained by the desirability function approach. The response surface plots were prepared to represent a function of two independent variables while keeping the other two independents variables at their optimal value.

The experimental data for gel strength, viscosity and TPA were measured three times. For these pair comparison between two groups, the t-test procedure was used and analyzed using of computer program SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Confidence level was set at $P \leq 0.05$.

Results and Discussion

Optimization of gelatin extraction process by response surface method

To fit a full response surface model, regression analysis was employed. Investigated responses include all linear (X_1, X_2, X_3, X_4), interaction (X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4), quadratic terms (X_1^2, X_2^2, X_3^2, X_4^2), and cubic (X_1^3, X_2^3, X_3^3, X_4^3). All insignificant terms (P>0.05) were eliminated to develop the fitted response surface model equations. The fitted models are shown in Table 2. Coefficients of correlation and determination was used to judge the quality of fit of the models. The quadratic model was suitable in this experiment to the response of Y. The high R^2 value of Y (0.9773) reflects the suitability of the model to represent the real relationships between the selected reaction parameters. Many statistical analysis methods were used for fitting the model, to judge the experimental error, the statistical significance of the terms in the model, and the suitability of the model. Table 3 shows how the adequacy of the model is acceptable in the present work through analysis of variance (ANOVA). The significance of the model is implied by the model's F-value of 26.36. The chance that such a large value of a "Model F-Value" could occur due to noise is only a 0.01%. Model terms are significant due to the values of "Prob > F" < 0.0500. X_1, X_2, X_4, X_1^2, X_2^2, X_3^2, X_4^2, X_1X_3, X_2^3, X_3^3 would be significant model terms in this case. Values of "Prob> F" >0.1000 indicate the model terms are not significant. The lack of fit is not significant relative to the pure error due to "Lack of Fit F-value" of 2.41. Non-significant lack of fit implies that the model is fit. Moreover, the values of adjusted R^2, predicted R^2, R^2, and adequate precision of this model are respectively 0.9403, 0.8400, 0.9773, and 15.691. The "Predicted R^2" of 0.8400 and the "Adjusted R^2" of 0.9403 are in reasonable agreement. The signal to noise ratio is reflected by "Adequate Precision" value. A ratio > 4 is desirable. Ratio of 15.691 in this experiment is an indication of an adequate signal. To navigate the design space, this model can be used. The following equation is obtained by RSM as the final response surface regression equation.

Table 3. ANOVA for response surface models

Source	Sum of squares	DF	Mean square	F Value	Prob > F
Model	4477.47	18	248.75	26.36	< 0.0001
X_1	108.14	1	108.14	11.46	0.0061
X_2	73.10	1	73.10	7.74	0.0178
X_3	30.88	1	30.10	3.27	0.0979
X_4	225.09	1	225.09	23.85	0.0005
X_1^2	972.82	1	972.82	103.08	< 0.0001
X_2^2	699.36	1	699.36	74.10	< 0.0001
X_3^2	637.61	1	637.61	67.56	< 0.0001
X_4^2	868.65	1	868.65	92.04	< 0.0001
X_1X_2	15.62	1	15.62	1.65	0.2247
X_1X_3	54.18	1	54.18	5.74	0.0355
X_1X_4	5.68	1	5.68	0.60	0.4542
X_2X_3	30.18	1	30.18	3.20	0.1013
X_2X_4	9.08	1	9.08	0.96	0.3478
X_3X_4	35.16	1	35.16	3.72	0.0798
X_1^3	4.32	1	4.32	0.46	0.5127
X_2^3	439.60	1	439.60	46.58	< 0.0001
X_3^3	77.94	1	77.94	8.26	0.0151
X_4^3	7.74	1	7.74	0.82	0.3846
Residul	103.81	11	9.44		
Lack of fit	77.11	6	12.85	2.41	0.1768
Pure error	26.70	5	5.34		
Cor total	4581.28	29			

X_1: Concentration of HCl (%), X_2: Pre-treatment time (h), X_3: Extraction time (h), X_4: Extraction temperature (°C)

$$Y = -174.60 + 37.01X_1 + 5.37X_2 - 7.14X_3 + 4.75X_4 - 8.65X_1X1 - 0.36X_2X_2 + 3.52X_3X3 - 0.05\ X_4X_4 + 0.12X_1X_2 + 1.23X_1X_3 + 0.04X_1X_4 + 0.11X_2X_3 + 6.28X_2X_4 - 0.06X_3X_4 + 0.30X_1X_1X_1 + 5.91X_2X_2X_2 - 0.38X_3X_3X_3 + 1.19X_4X_4X_4$$

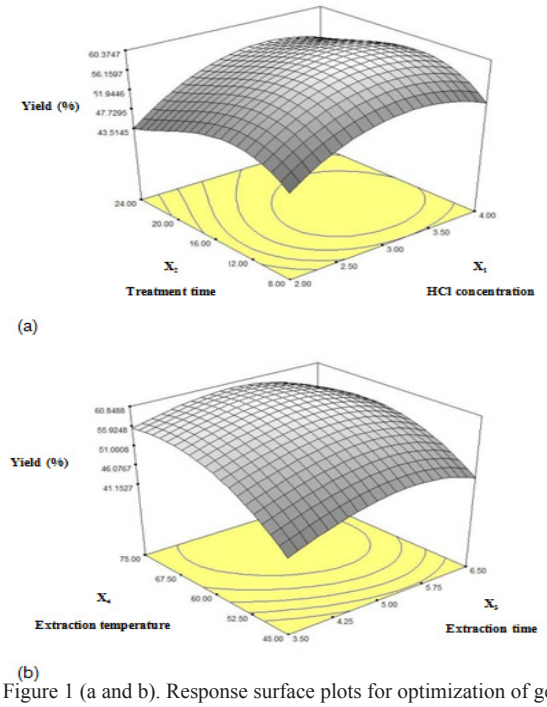

Figure 1 (a and b). Response surface plots for optimization of gelatin extraction from catfish (*Clarias gariepinus*) bone

Table 4. Proximate compositions of raw catfish (*Clarias gariepinus*)

Composition	Fish bone % (wet weight)	Fish bone gelatin % (wet weight)
Moisture	44.48 ± 1.14^{b}	11.43 ± 0.54^{a}
Protein	30.77 ± 0.88^{b}	81.75 ± 0.83^{c}
Lipid	6.55 ± 0.34^{b}	0.95 ± 0.40^{a}
Ash	12.62 ± 0.73^{a}	5.60 ± 0.26^{a}

Effects of independent variables on yield of hydroxyproline are visualized by 3D- views of response surface plots and respective contour plots in Figure 1a and b. The plots are representations of two factors at a time by holding the third and fourth factor at a fixed level (middle level). Both plots are of convex form with a peak maximum for extraction yield which can be used to find the optimal values for independent variables. The corresponding values of independent variables of HCl concentration (X_1), treatment time (X_2), extraction time (X_3), and extraction temperature (X_4) were read while dependent variable was fixed at its maximum. The optimal values were HCl concentration (X_1) 3.35%, treatment time(X_2) 14.5hrs, extraction time(X3) 5.2hrs, and extraction temperature(X_4) 67.23°C. The predicted value of Y was 61.81% with a desirability of 0.977, while actual experimental results repeated three times under optimal conditions were 60.54%.

Proximate composition

The proximate composition of raw catfish bone and extracted gelatin are shown in Table 4. The protein, lipid and ash content of catfish bone gelatin were found to be 81.75%, 0.95% and 5.60%, respectively. Muyonga *et al*. (2004) elucidated that the protein content of the collagenous material represented the maximum possible yield of gelatin expected from them. The ash content of the catfish bone gelatin (5.60%) is quite high which may be due to the short period of acidification or the low concentration of the applied acid. For future studies, measuring the bone ash content before extraction of gelatin is recommended. Other differences are less moisture and fewer lipids in bone gelatin (Table 4) which can be due to the increased of calcification in bones (Taheri *et al*., 2009).

Yield of gelatin

The catfish bone gelatin yield was 17.52% (w/w). The low yield could be due to incomplete hydrolysis of the collagen resulting in loss of extracted collagen (Jamilah and Harvinder, 2001). The extraction of collagen rod is done in acid and solubilized without changing its original triple-helix structure. The following thermal treatments cut hydrogen and covalent bonds, which destabilizes the triple helix through a helix-to-coil transition, converting it into gelatin (Montero and Gomez-Guillen, 2000). Different gelatin yield values extracted from skins and bones of other fish are reported in the literature, such as Dover sole 8.3%, megrim 7.4%, hake 6.5%, cod 7.2% (Gómez-Guillén *et al*., 2001); red tilapia 7.8%, black tilapia 5.4% (Jamilah and Harvinder, 2002); big eye snapper 6.5% and brown stripe red snapper 9.4% (Jongjareonrak *et al*., 2006); short fin scad 7.3% (Cheow *et al*., 2007). It has been concluded that the variation in these values are due to differences in proximate composition of skins and bones, amount of soluble components in the skins and bones and the collagen content. These properties vary in different species of the fish, as well as the variants of the extraction technique (Songchotikunpan *et al*., 2008).

Gel strength

One of the most significant physical properties of gelatin is the gel strength. Gel strength of fish gelatin is typically less than mammalian gelatin (Gilsenan and Ross-Murphy, 2000). Table 5 shows the gel strengths of the extracted catfish bone gelatin and bovine gelatin. Bovine gelatin has significantly higher gel strength (300 g) than catfish bone gelatin (230.25 g). This was probably due to lower gel forming capability of this gelatin because of the shorter length of this gelatin molecule chains. The gel strength of catfish

Table 5. Gel strength, viscosity and TPA of extracted gelatin from catfish (*Clarias gariepinus*) bone and bovine

		Catfish (*Clarias gariepinus*) bone gelatin	Bovine gelatin
Gel strength (g)		230.25 ± 6.96[b]	300.00 ± 20.11[a]
Viscosity (mPa.s)		4.64 ± 0.51[a]	3.17 ± 0.44[b]
TPA	Hardness	201.48 ± 10.54[b]	346.35± 27.23[a]
	Cohesiveness	0.89 ± 0.00[a]	0.92 ± 0.00[a]
	Springiness	1.09 ±0.18[a]	0.97 ± 0.06[a]
	Chewiness	200.49 ± 38.25[b]	310.75 ± 10.95[a]
	Gumminess	181.23 ± 10.23[b]	318.88 ± 26.39[a]

[a,b] Means ± standard deviation of triplicate determinations. Means in the same raw with different superscript letters are significantly different (P≤0.05)

Table 6. Amino acid composition of catfish (*Clarias gariepinus*) bone gelatin (residues per 1000 total amino acid residues)

Amino acids	Number of residues/1000
Alanine	91
Arginine	60
Aspartic acid	65
Cystine	0
Glutamic acid	91
Glycine	212
Hydroxyproline	53
Isoleucine	15
Leucine	25
Lysine	35
Methionine	77
Phenylalanine	29
Proline	90
Serine	38
Threonine	39
Tryrosine	6
Valine	25
Total	1000
Hydroxyproline+Proline	143

Data presented as means ± standard deviation of duplicated determination. Correspond to mean values. Standard deviations were in all cases lower than 1%

bone gelatin (183 g) was greater than gelatin such as lizardfish bone (135 g) and skin (159 g) (Taheri *et al*., 2009), shark cartilage (111.9 g) (Cho *et al*., 2004) cod (90g) and hake (110 g) (Gómez-Guillén *et al*., 2002), alaska pollock (98 g) (Zhou and Regenstein, 2005) and salmon (108 g) (Arnesen and Gildberg, 2006), but lower than gelatin from species such as yellowfin tuna skin (426 g) (Cho *et al*., 2005), nile tilapia skin (328 g) (Songchotikunpan *et al*., 2008) and lizardfish scales (268 g) (Wangtueai and Noomhorm, 2009). Furthermore gel strength is influenced by many factors including molecular weight distribution of gelatin and gelatin concentration (Jamilah and Harvinder, 2001), size of protein chains and amino acid compositions (Muyonga *et al*., 2004).

Texture profile analysis

Table 5 reveals the TPA compression test on catfish bone gelatin and bovine gelatin. Gumminess is the product of hardness multiplied by cohesiveness. Chewiness is the product of three values for hardness multiplied by cohesiveness multiplied by springiness (Wangtueai and Noomhorm, 2009). Gel hardness of catfish bone gelatin was found to be lower than bovine gelatin (Table 5). Catfish bone gelatin and bovine gelatin had a springiness of 1.09 and 0.97; and a cohesiveness of 0.89 and 0.92 respectively (Table 5). Cohesiveness and springiness of catfish bone gelatin were found to possess similar characteristics to bovine gelatin (P>0.05). However, catfish bone gelatin gave considerably lower (P < 0.05) hardness, gumminess and chewiness than bovine gelatin, both compared at the same concentrations (6.67%).

Viscosity

The second important physical characteristic of the gelatin is the viscosity. Table 5 compares the shear viscosity of catfish bone gelatin with bovine gelatin. Catfish bone gelatin has significantly higher viscosity (4.64 mPa.s) than bovine gelatin (3.17 mPa.s). Furthermore, the viscosity of catfish bone gelatin (4.64 mPa.s) is higher than viscosity those reported for rainbow (3.2 mPa.s) (Tabarestani *et al*., 2010) and red tilapia (1.73 mPa.s) (See *et al*., 2010) but much lower than those for lizardfish scale (7.5 mPa.s) (Wangtueai and Noomhorm, 2009) and hoki skin (10.8 mPa.s) (Mohtar *et al*., 2010). In comparison between shear viscosity values and gel strength of extracted gelatins, it was noted that extracted gelatins, which possessed higher gel strength showed a lower shear viscosity and vice versa. The viscosity of gelatin solutions is partially controlled by molecular weight and polydispersity. The viscosity of catfish bone gelatin is in mid range values since the viscosity of commercial gelatins is in the range of 2 to 7 mPa.s in most cases and may goes up to 13 mPa.s for specialized cases (Johnston-Banks, 1990).

Amino acid composition

Table 6 presents the amino acid composition of catfish bone gelatin. The imino acid (hydroxyproline and proline) and glycine contents of the catfish bone gelatin were 143 residues per 1000 residues and 212 residues per 1000 residues, respectively. The contents of glycine and imino acid are important for gel strength. The mammalian gelatins contain a high composition of these three amino acids (Wangtueai and Noomhorm, 2009), especially hydroxyproline

and proline, which are related to the property of gelling. A low amount of imino acids indicates a poor gelling ability (Wangtueai and Noomhorm, 2009).

Isoionic point

Extracted gelatin from catfish bone had higher isoionic points (8.71) than bovine gelatin (5.35), which were also close to the isoionic point of collagen (9.0-9.4). This was due to shorter period of acidic pretreatment (normally 10-72 hours) in which of deamidation of asparagines and glutamine less occurs. A type A gelatin is produced by acid processing, producing isoionic point ranging from 7 to 9 meanwhile a type B gelatin such as commercial bovine gelatin normally has lower isoionic point. This might be due to the prolonged alkaline pretreatment (7 days to 3 months).

Conclusions

This study revealed the potential of catfish (*Clarias gariepinus*) bone as raw material for gelatin production, giving relatively high protein content which contributed to high viscosity and gel strength. The HCl concentration and treatment time along with extraction temperature and extraction time were found to significantly affect hydroxyproline yield. According to the RSM model, the optimum conditions for gelatin extraction was obtained using 3.35% HCl for 14.5 h and hot water extraction at 67.23°C for 5.2 h. Gelatin extracted from catfish bone was proven to exhibit a higher and comparable characteristics to bovine gelatin hence could be used in food industries as a replacement for mammalian gelatin.

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References

AOAC. 2000. Official methods of analysis. 15th edn. Virginia: Association of Official Analytical Chemists, Inc.

Arnesen, J.A. and Gildberg, A., 2006. Extraction of muscle proteins and gelatin from cod head. Process Biochemistry 41: 697–700.

Arteaga, G. E., Li-Chan, E., Vazquez-Arteaga, M. C. and Nakai, S. 1996. Systematic experimental designs for product formula optimization. Trends in Food Science and Technology 5: 243–254.

BSI (British Standards Institution) 1975. Methods for sampling and testing gelatin (physical and chemical methods). London: BSI.

Cho, S. M., Kwak, K.S., Park, D.C., Gu, Y.S., Ji, C.I., Jang, D.H., Lee, Y.B and Kim, S.B. 2004. Processing optimization and functional properties of gelatin from shark (*Isurus oxyrinchus*) cartilage. Food Hydrocolloids 18: 573-579.

Cho, S.M., Gu, Y.S., and Kim, S.B. 2005. Extracting optimization and physical properties of yellowfin tuna (*Thunnus albacares*) skin gelatin compared to mammalian gelatins. Food Hydrocolloids 19: 221-229.

Cole, C.G.B., 2000, Gelatin, in Encyclopedia of Food Science and Technology, Frederick, J. Francis (ed) (John Wiley and Sons, New York, USA), pp. 1183–1188. (John Wiley and Sons, New York, USA).

Demars LL, Ziegler GR. 2001. Texture and structure of gelatin/pectin-based gummy confections. Food Hydrocolloids 15: 643-53.

Department of Fisheries Malaysia. 2007. Estimated aquaculture production from freshwater culture system by species, 2007. *http://www.dof.gov.my/c/document_library/get_file?uuid=07ab7a5d-908b-4c15-9927-874ff729490e&groupId=10131*.

Gilsenan, P.M. and Ross-Murphy, S.B. 2000. Rheological characterisation of gelatins from mammalian and marine sources. Food Hydrocolloids 14: 191-195.

Gómez-Guillén, M.C. and Montero, P. 2001. Extraction of gelatin from megrim (*Lepidorhombus boscii*) skins with several organic acids. Journal of Food Science 66: 213-216.

Gómez-Guillén, M.C., Turnay, J., Fernández-Diaz, M.D., Ulmo, N., Lizarbe, M.A. and Montero, P. 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocolloids 16: 25-34.

Gómez-Guillén, M.C., Giménez, B., López-Caballero, M.E. and Montero, M.P. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources. Food Hydrocolloids 25(8): 1813-1827.

Jamilah, B. and Harvinder, K.G. 2001. Properties of gelatins from skins of fish-black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*). Food Chemistry 77: 81-84.

Johnston-Banks, F. A. 1990. Gelatin. In Harris, P. (Eds.). Food Gel, p. 233-289. New York: Elsevier Applied Sciences.

Jongjareonrak, A., Benjakul, S., Visessanguan,W., Prodpran, T. and Tanaka, M. 2006. Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye snapper. Food Hydrocolloids 20: 492-501.

Kasankala, L.M., Xue, Y., Weilong, Y., Hong, S.D., and He, Q. 2007. Optimization of gelatine extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technology 98: 3338-3343.

Liu, H.Y., Li, D., and Guo, S.D. 2008. Extraction and properties of gelatin from channel catfish (*Ietalurus punetaus*) skin. LWT-Food Science and Technology 41: 414-419.

Muyonga, J.H., Colec, C.G.B. and Duodub, K.G. 2004. Extraction and physicochemical characterisation of Nile perch (*Lates niloticus*) skin and bone gelatine. Food Hydrocolloids 8: 581-592.

Mohtar, N.F., Perera, C. and Quek, S.Y. 2010. Optimisation of gelatine extraction from hoki (*Macruronus novaezelandiae*) skins and measurement of gel strength and SDS–PAGE. Food Chemistry 122: 307-313.

Myers, R. H., and Montgomery, D. C. 1995. Response surface methodology: Process and product optimization using designed experiments. New York, USA: John Wiley-Interscience Publication.

See, S.F., Hong, P.K., Ng, K.L., Wan Aida, W.M. and Babji, A.S. 2010. Physicochemical properties of gelatins extracted from skins of different freshwater fish species. International Food Research Journal 17: 809-816.

Tabarestani, S.H., Maghsoudlou, Y., Motamedzadegan, A. and Mahoonak, S.A.R. 2010. Optimization of physic-chemical properties of gelatin extracted from fish skin of rainbow trout (*Onchorhynchus mykiss*). Bioresource Technology 101: 6207-6214.

Songchotikunpan, P., Tattiyakul, J. and Supaphol, P. 2008. Extraction and electrospinning of gelatin from fish skin. International Journal of Biological Macromolecules 42: 247-255.

Taheri, A., Abedian Kenari, A.M., Gildberg, A. and Behnam, S. 2009. Extraction and Physicochemical Characterization of Greater Lizardfish (*Saurida tumbil*) Skin and Bone Gelatin. Journal of food science 74: E160-E165.

Wangtueai, S. and Noomhorm, A. 2009. Processing optimization and characterization of gelatin from lizardfish (*Saurida* spp.) scales. LWT - Food Science and Technology 42: 825–834.

Yang, H.,Wang, Y., Jiang, M., Oh, J.H., Herring, J., and Zhou, P. 2007. 2-Step optimization of the extraction and subsequent physical properties of channel catfish (*Ictalurus punctatus*) skin gelatin. Journal of Food Science 72: C188-C195.

Zhou, P., and Regenstein, J.M. 2005. Effects of alkaline and acid pretreatments on Alaska Pollock skin gelatin extraction. Journal of Food Science 70: C392-C396.