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Processing Optimization and Characterization of Gelatin from Catfish (*Clarias gariepinus*) Skin

(Pengoptimuman Pemprosesan dan Pencirian Gelatin daripada Kulit Ikan Keli (*Clarias gariepinus*))

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ABSTRACT

*The extraction of catfish (*Clarias gariepinus*) skin gelatin was optimized by using response surface methodology (RSM) employing a central composite design (CCD). RSM with 3-factors, 5-levels CCD was carried out for the optimization. The independent variables were suggested which include NaOH concentration (0.07-0.23 mol/L), acetic acid concentration (0.04-0.14 mol/L) and extraction temperature (40-80°C) with the percentage of hydroxyproline recovery (Y) as dependant variable. A maximum Y of 65.32% for gelatin processing was obtained using a combination of 0.13 mol/L NaOH and 0.09 mol/L acetic acid for 1 h, followed by a hot-water extraction at 64.92°C for 3 h. The results indicated a high protein content (88.46 g/100 g) in the extracted gelatin with a viscosity of 3.45 mPa.s, 286.71 g gel strength and 173 residues per 1000 residues of imino acids (proline and hydroxyproline). Furthermore, the gelatin from catfish also showed a relatively good instrumental texture quality according to texture profile analysis (TPA).*

Keywords: Bovine gelatin; catfish; central composite design; fish skin gelatin; response surface methodology

ABSTRAK

*Pengekstrakan gelatin daripada kulit ikan keli (*Clarias gariepinus*) dioptimumkan dengan menggunakan kaedah sambutan permukaan (RSM) menggunakan reka bentuk komposit tengah (CCD). RSM dengan faktor 3-, 5-aras CCD telah digunakan untuk pengoptimuman. Parameter tak bersandar yang dicadangkan termasuk kepekatan NaOH (0.07-0.23 mol/L), kepekatan asid asetik (0.04-0.14 mol/L) dan suhu pengekstrakan (40-80°C) dengan peratusan pemulihan hidroksiprolin (Y) sebagai parameter bersandar. Nilai maksimum Y (65.32%) untuk pemprosesan gelatin telah diperoleh dengan menggunakan kombinasi 0.13 mol/L NaOH dan 0.09 mol/L asid asetik untuk 1 jam dan diikuti dengan pengekstrakan menggunakan air panas pada 64.92°C selama 3 jam. Keputusan menunjukkan ekstrak gelatin mengandungi kandungan protein yang tinggi (88.46 g/100 g) dengan nilai kelikatan 3.45 mPa.s, nilai kekuatan gel 286.71 g dan 173 sisa per 1000 sisa asid imino (prolin dan hidroksiprolin). Selain itu, gelatin daripada ikan keli juga mempunyai kualiti tekstur yang baik menerusi analisis profil tekstur (TPA).*

Kata kunci: Gelatin kulit ikan; ikan keli; kaedah permukaan respons; reka bentuk komposit tengah

Introduction

Gelatin is considered an important raw material in various industries such as photography, pharmaceutical and cosmetic (Wangtueai & Noomhorm 2009). In the food industry, gelatin is used extensively in products like candies, desserts, jelled meats, bakery products, dairy and ice cream. Gelatin is obtained from collagen; therefore, the properties of the extracted gelatin depend on the type and source of collagen. Typically, gelatin has been produced from skin of porcines, as well as bones and skin of bovines (Hinterwaldner 1977; Ward & Courts 1977). There are religious issues related to gelatin produced from porcine or bovine for Muslims, Hindus and Jews. Fish gelatin is a substitute to the mammalian gelatin, acceptable as a food enhancer by these religious groups (Haug et al. 2004). Frequently, it may be necessary to differentiate between gelatins from different sources not only for safety and religious reasons, but because it has been reported that most gelatin-allergic patients develop allergic reactions to bovine and porcine gelatin, but do not react to fish gelatin (Sakaguchi et al. 2000). Despite labeling precautions, bovine and porcine gelatins pose a high degree of risk for sensitized patients because they are often present in commercial foods and food ingredients, due to cross-contamination during processing. It was reported that skin and bones of fish comprise of nearly 30% of fish after removing the edible parts (Taheri et al. 2009). Therefore, research activities focusing on developing techniques to obtain fish gelatin as a replacement for mammalian and porcine sources are of big interests (Wangtueai & Noomhorm 2009).

The extraction of gelatin from skins and bones of different fish species have been widely investigated (Cheow et al. 2007). Studies on gelatin extraction of skin have been reported which include Alaska pollock skin (Zhou & Regenstein 2004), megrim skin (Montero & Gomez-Guillen 2000), grass carp skin (Kasankala et al. 2007), lizardfish skin and bone (Taheri et al. 2009),

channel catfish skin (Liu et al. 2008: Yang et al. 2007), shark cartilage (Cho et al. 2004), yellow fin tuna skin (Cho et al. 2005) and grey triggerfish (Jellouli et al. 2011).

In this study, *Clarias* catfish (*Clarias gariepinus*) was chosen for gelatin extraction of its skin. 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. 2002). Until now, gelatin from the skins of African 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 21891.55 metric tons. The sale of catfish produced in Malaysia in year 2007 earned RM107 million out of the total aquaculture fish production of RM481 million. Catfish skin, comprising about 5% of the whole fish, has become an interesting raw material for gelatin production.

Response surface methodology (RSM) has shown effectiveness in the optimization and monitoring of food processes (Wangtueai & Noomhorm 2009). Response surface methodology (RSM) is a set of statistical techniques used to regulate the effects of multiple parameters in a process and to optimize different processes. In this mathematical modelling technique independent and dependent variables are correlated to establish a regression equation which describes the interrelations between output properties and input variables (Yang et al. 2007). The objectives of this study were to determine the critical variables for extraction of catfish skin gelatin and the optimal extraction parameters to obtain maximum yield of hydroxyproline recovery using RSM. Furthermore, the physicochemical properties of catfish gelatin were compared with bovine gelatin.

Materials and Methods

MATERIALS AND PREPARATION

Catfish used in this process were obtained from Penang, Malaysia. The fish were transferred to the laboratory and the skins were removed manually. After filleting, the fish skins were washed thoroughly to remove the attached meats. The washed skins were packed in Zip lock plastic bag (1 kg/bag) after being chopped to small pieces (2 cm × 2 cm). The fish skins were kept frozen at -20°C until further use. Commercial gelatin from bovine skin (type B) was bought from Sigma (Sigma–Aldrich Chemical Co., USA), which was selected for comparison for the physicochemical characteristics with the fish skin gelatin. All reagents used, were of analytical grade.

GELATIN EXTRACTION

After treating cleaned skins (30 g) for 60 min with NaOH of varying OH^- concentrations (0.07-0.23 mol/L), they were drained and washed 3 times with tap water. Then, the samples were treated for 60 min with acetic acid of different concentrations (0.04-0.14 mol/L), based on the design. The samples were drained and rinsed 3 times with tap water. The preparation process above was carried out at 4°C. Finally, samples were mixed with distilled water (the ratio of skin/water, 1/8 w/v) and gelatin was extracted at different temperatures (40-80°C) each time for 3 h. Finally, the gelatin solution was filtered through cheesecloth with 4 layers and then centrifuged at 10000 g for 20 min. Yield of hydroxyproline recovery of gelatin extracted was determined by the method of ISO (1978).

PROXIMATE COMPOSITON

The lipid, ash and moisture content of raw catfish skin and catfish skin gelatin were measured according to AOAC (1999). Protein content was measured by Kjeldahl method whereas for calculation of crude protein content, a nitrogen conversion factor of 6.25 and 5.4 were used on raw fish skin and the extracted gelatin, respectively (Muyonga et al. 2004).

GELATIN YIELD

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

$$\text{Yield of gelatin (g/100 g)} = (\text{weight of dried gelatin (g)} / \text{wet weight of fresh skin (g)}) \times 100.$$

GEL STRENGTH

Based on British Standard 757: 1975 method (BSI 1975), a 6.67 g/100 mL gelatin solution was prepared by mixing distilled water with 7.5 g of the extracted gelatin. To allow gelatin to swell, the mixture was left for 30 min at room temperature. The mixture was then heated for 20 min at 65°C to completely dissolve gelatin and then kept for 16-18 h at 4ºC in a refrigerator. The gel strength was measured by a Texture Analyzer Stable Micro (TAXT2, UK) System equipped with a plunger (1.27 cm in diameter). The measurements were carried out in triplicate and at the penetration depth of 4 mm. The maximum force (g) was recorded at a rate of 0.5 mm/s.

VISCOSITY

The samples used for gel strength determination were melted in a water bath maintained at 45ºC. 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.

TEXTURE PROFILE ANALYSIS

For texture profile analysis (TPA), the same gelatin samples as those taken for gel strength were used. Texture profile analysis test was carried out by using a TA.XTPlus Texture Analyzer (UK) with a 75-mm-dia plate. A reasonable compression value of 40% for TPA was assigned based on the preliminary experiment and other researches (DeMars & Ziegler 2001). The test was performed according to Yang et al. (2007). The results were taken as the average of 3 replicates of the same lot. Textural parameters such as springiness, cohesiveness, hardness, gumminess and chewiness were measured from the force-time curve of the texture profile by using the method as described previously.

AMINO ACID COMPOSITION

Gelatin samples were hydrolyzed in 6 mol/L HCl at 110ºC for 16 h. The hydrolysate was dissolved in deionized water and filtered. The amino acid composition was examined by a high performance liquid chromatography (HPLC), equipped with a Waters 410 Scanning Flourescence and AccQ Tag column (3.9 × 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).

EXPERIMENTAL DESIGN

RSM with 3-factors, 5-levels Central Composite Design (CCD) was implemented to optimize gelatin extraction. Concentration of NaOH (factor X_1, mol/L), concentration of acetic acid (factor X_2, mol/L) and extraction temperature (factor X_3, °C) were chosen as the independent variables along with percentage of hydroxyproline recovery as the dependent variable. After the set up of conditions for the desired range for the independent variables, the RSM software would supply many groups of optimizations (Tables 1 & 2).

TABLE 1. Independent variables and their levels in the 3-factor, 5-level central composite design for optimizing the extraction condition of catfish (*Clarias gariepinus*) skin gelatin

Independent variable	Symbol	Level				
		–2	–1	0	1	2
Concentration of NaOH (mol/L)	X1	0.07	0.1	0.15	0.2	0.23
Concentration of acetic acid (mol/L)	X2	0.04	0.06	0.09	0.12	0.14
Extraction temperature (˚C)	X3	40	50	60	70	80

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

Standard order	Independent variable			Y (Exp)	Y(Pred)
	X_1	X_2	X_3		
1	-1	-1	-1	53.79	52.68
2	1	-1	-1	54.47	53.38
3	-1	1	-1	49.20	47.80
4	1	1	-1	51.46	50.90
5	-1	-1	1	61.12	60.91
6	1	-1	1	54.51	55.13
7	-1	1	1	62.92	63.23
8	1	1	1	59.51	59.84
9	-2	0	0	60.12	61.18
10	2	0	0	58.87	58.92
11	0	-2	0	58.56	59.26
12	0	2	0	58.73	59.13
13	0	0	-2	39.38	41.47
14	0	0	1	56.91	55.91
15	0	0	0	64.11	64.11
16	0	0	0	63.24	64.11
17	0	0	0	62.82	64.11
18	0	0	0	66.05	64.11
19	0	0	0	64.54	64.11
20	0	0	0	64.08	64.11

X_1: concentration of NaOH (mol/L), X_2: concentration of acetic acid (mol/L), X_3: extraction temperature (°C), Y: yield of hydroxyproline recovery

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 second-order polynomial equation was chosen to represent the experimental data, which was obtained using response surface regression:

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

where Y is the dependent variable, 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).

As three parameters were varied, 10 β-coefficients had to be estimated which included coefficients for the three main effects, three quadratic effects, three interactions and one constant. It is assumed that the estimated behavioural model of both dependent variables was described by a second-order polynomial equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

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

The experimental data of gel strength, viscosity and TPA were measured three times repeatedly. For the pair comparison between two groups, t-test procedure was used and analyzed by SPSS statistical program (Version 16.0) (SPSS Inc., Chicago, IL).

Results and Discussion

Optimization of the gelatin processing

To fit a full response surface model for all investigated responses including linear (X_1, X_2, X_3), interaction (X_1X_2, X_1X_3, X_2X_3) and quadratic terms (X_1^2, X_2^2, X_3^2), regression analysis was employed. All insignificant terms ($p > 0.05$) were eliminated to calculate the fitted response surface model equations. The fitted models are shown in Table 3. The response surface regression equation gained by RSM is:

$$Y = -175.68 + 317.42X_1 - 73.27X_2 + 6.91X_3 - 574.35X_1X_1 - 1930.51X_2X_2 - 0.054X_3X_3 + 398.38X_1X_2 - 3.24\,X_1X_3 + 5.99X_2X_3$$

The models were judged for the quality of fit by coefficients of correlation and determination. The quadratic model was suitable to the response of Y in this experiment with high R^2 values 0.9760, which indicates the appropriateness of the model to represent the real relationships among all of the selected reaction parameters. Various statistical analysis methods were used to judge the experimental error, the statistical significance of the terms in the model and the suitability of the model in order to fit judge the fitting of the model. Table 3 shows how the adequacy of this model is justified in the present work through analysis of variance (ANOVA). The Model *F*-value of 45.09 indicates that the model is significant. The chance that a 'Model *F*-Value' with such a large value could occur due to noise is only 0.01%. Values of 'Prob > *F*' < 0.0500 indicate that the model terms are significant. The X_3, X_1^2, X_2^2, X_3^2, X_1X_3, X_2X_3 are significant model terms in this case. Values of 'Prob > *F*' > 0.1000 imply that the model terms are not significant. 1.96 as the 'Lack of Fit *F*-value' shows that the lack of fit is not significant relative to the pure error. A non-significant lack of fit is a good indicator that the model is fit. Moreover, the values of R^2, predicted R^2, adjusted R^2 and adequate precision for this model are 0.9760, 0.8669, 0.9543 and 23.306, respectively. The 'Predicted R^2' of 0.8669 and the 'Adjusted R^2' of 0.9543 are in reasonable agreement. 'Adequate Precision' is a measure of the signal to noise ratio. A ratio of 4 or greater is desirable. Ratio of 23.306 in this experiment indicates an adequate signal.

For the optimization of gelatin extraction, three independent variables were selected, which included a concentration of NaOH (X_1) 0.13 mol/L, acetic acid concentration (X_2) 0.09 mol/L and extraction temperature (X_3) 64.92°C. The predicted value of Y was 65.32% with a desirability of 0.973, while actual experimental results repeated three times under optimal conditions were 64.27%.

Figure 1(a) and 1(b) shows the three-dimensional response surface plots of the independent variables (X_1, X_3) and (X_2, X_3) against the dependent variable (Y). The plots are representations of two factors at a time by holding the third 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 NaOH concentration (X_1), acetic acid concentration (X_2), extraction temperature (X_3) were read while dependent variable was fixed at its maximum.

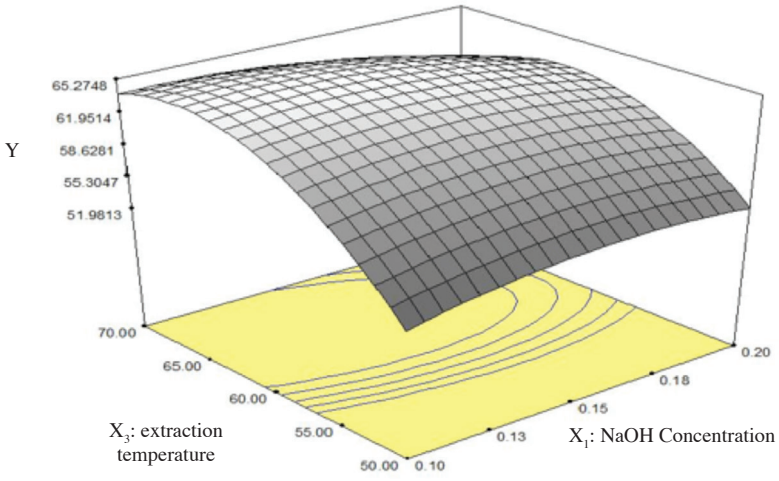
Proximate composition

Table 4 compares the proximate composition of catfish skin and catfish skin gelatin. The catfish skin contained high protein and lipid content on wet basis (41.31 g/100 g and 7.26 g/100 g, respectively) but low in ash (0.68 g/100 g). Muyonga et al. (2004) reported that the protein content in the collagenous material represents the possible maximum yield of the gelatin. The fish skin composition has been reported for Nile tilapia skin (30.6 g/100 g protein, 1.1 g/100 g lipid and 2.1 g/100 g ash) (Songchotikunpan et al. 2008) and lizardfish skin (26.6 g/100 g protein, 2.4 g/100 g fat and 1.44 g/100 g ash) (Taheri et al. 2009). Furthermore,

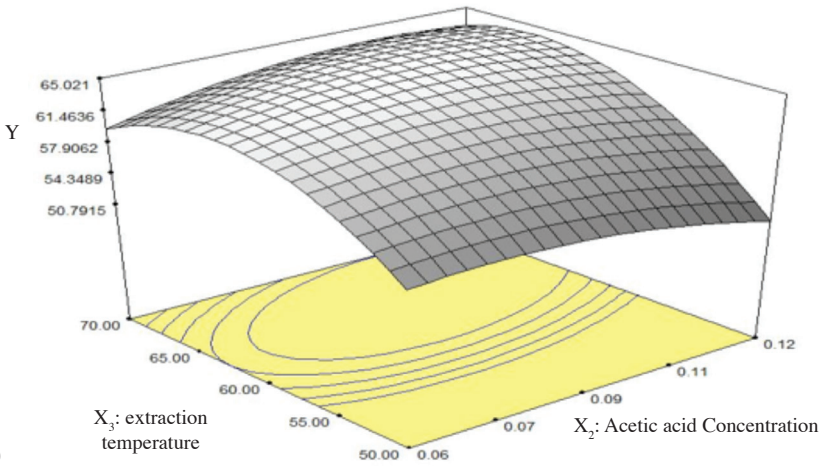
TABLE 3. Analysis of variance (ANOVA) for response of the dependent variable (Y, %)

Source	Sum of square	DF	Mean square	F Value	Prob > F
Model	765.54	9	85.06	45.09	< 0.0001
X_1	6.15	1	6.15	3.26	0.1011
X_2	0.02	1	0.02	0.01	0.9197
X_3	251.72	1	251.73	133.45	< 0.0001
X_1^2	29.71	1	29.71	15.75	0.0026
X_2^2	43.50	1	43.50	23.06	0.0007
X_3^2	428.00	1	428.00	226.89	< 0.0001
$X_1 X_2$	2.86	1	2.86	1.51	0.2466
$X_1 X_3$	20.99	1	20.99	11.13	0.0075
$X_2 X_3$	25.88	1	25.88	13.72	0.0041
Residual	18.86	10	1.89	1.96	0.2393
Lack of fit	12.49	5	2.50		
Pure error	6.38	5	1.28		
Total	784.41	19			

X_1: concentration of NaOH (mol/L), X2: concentration of acetic acid (mol/L), X3: extraction temperature (°C)



(a)



(b)

FIGURE 1. (a) and (b). Response surface plots for optimization of gelatin extraction from catfish (*Clarias gariepinus*) skin. X_1 (concentration of NaOH, mol/l), X_2 (acetic acid concentration, mol/l), X_3 (extraction temperature, ˚C) and Y (extraction yield, Y%)

TABLE 4. Proximate composition of raw catfish (Clarias gariepinus) skin and extracted gelatin

Composition (g/100 g wet weight)	Catfish skin	Catfish skin gelatin
Moisture	50.86 ± 1.16	10.56 ± 0.89
Protein	41.31 ± 0.66	88.46 ± 0.80
Lipid	7.26 ± 0.45	0.74 ± 0.35
Ash	0.68 ± 0.25	0.48 ± 0.50

Data presented as means ± standard deviation of triplicate determinations

the work done by Mohtar et al. (2010) on hoki skins also showed fish skin could be used as collagenous material for gelatin extraction. The alkaline pre-treatment process of gelatin extraction is used to destroy certain cross-linked chemicals that are still presence in the collagen and to remove impurities and unwanted materials (Ward & Courts 1977). However, gelatin from catfish skin contained a considerably high protein (88.46 g/100 g) and low in lipid (0.74 g/100 g) and ash content (0.48 g/100 g). Low ash content contributes to a high quality gelatin (Jones 1977).

GELATIN YIELD

The yield of catfish skin gelatin was 21.79 g/100 g. The low yield could be due to incomplete hydrolysis of the collagen resulting in loss of extracted collagen (Jamilah & Harvinder 2002). Fish skin is a source of highly soluble collagen, holding low concentration of inter- and intra-chain non-reducible cross-links. Thus, a mild acid pre-treatment should usually be used on fish skins (Montero & Gomez-Guillen 2000). The extraction of collagen rod is done in acid and solubilized without changing the 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 & Gomez-Guillen 2000). Different gelatin yield values extracted from skins of other fish are reported in the literature, e.g. sin croaker (14.3 g/100 g) and shortfin scad (7.25 g/100 g) (Cheow et al. 2007), bigeye snapper (6.5 g/100 g) and brownstripe red snapper (9.4 g/100 g) (Jongjareonrak et al. 2006), Atlantic salmon (15.3 g/100 g) and cod (11.8 g/100 g) by a 2 step extraction (Arnesen & Gildberg 2007). It has been concluded that the variation in these values are due to differences in proximate composition of skins, amount of soluble components in the skins and the collagen content. These properties vary in different species and with the age of the fish, as well as the variants of the extraction technique (Songchotikunpan et al. 2008). Combinations of the two pre-treatments were used in this study. The alkaline and acidic pre-treatments is proved to be effective on removing non-collagenous proteins with minimum collagen loss by destroying certain cross linkage chemicals present in the collagen with least breakage of peptide bonds. This process was favourable in causing a large amount of swelling in fish skin, resulting in a high gelatin yield and good gel strength (Zhou & Regenstein 2005).

GEL STRENGTH

Gel strength is one of the most important physical properties of the gelatin (Cheow et al. 2007). There was no significant difference (p>0.05) in gel strength of catfish skin gelatin and bovine gelatin, indicating a similar quality of gel strength possessed by both gelatins (Table 5). The gel strength of catfish skin gelatin (286.71 g) was much greater than gelatin from sin croaker skin (124 g) and shortfin scad skin (176 g) (Cheow et al. 2007), cod (90 g) and hake (110 g) (Gómez-Guillén et al. 2002), Alaska pollock (98 g) (Zhou & Regenstein 2005) and salmon (108 g) (Arnesen & Gildberg 2007), but lower than gelatin from species such as yellowfin tuna skin (426 g) (Cho et

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

	Catfish skin gelatin	Bovine gelatin
Gel strength (g)	286.71 ± 15.57[a]	300.00 ± 20.11[a]
Viscosity (mPa.s)	3.45 ± 0.13[a]	3.17 ± 0.44[a]
TPA		
Hardness	361.74±10.3[a]	346.35± 27.23[a]
Cohesiveness	0.99 ± 0.04[a]	0.92 ± 0.00[b]
Springiness	0.99 ±0.01[a]	0.97 ± 0.06[a]
Chewiness	357.59 ± 12.73[a]	310.75 ± 10.95[a]
Gumminess	360.72 ± 14.25[a]	318.88 ± 26.39[b]

a,b, means ± standard deviation of triplicate determinations. Means in the same row with different superscript letters are significantly different (p≤ 0.05)

al. 2005) and Nile tilapia (328 g) (Songchotikunpan et al. 2008). The variation of gel strength is correlated to amino acid composition and size of protein chain (Muyonga et al. 2004), also gelatin concentration and molecular weight distribution (Ockerman & Hansen 1988).

VISCOSITY

The second most important physical property is the viscosity of a gelatin (Jamilah & Harvinder 2002). The viscosity of gelatin solution is partially controlled by molecular weight and polydispersity. Table 5 compares the shear viscosity of the extracted gelatin from catfish skin and bovine gelatin. There was no significant difference (p>0.05) in viscosity of catfish skin gelatin and bovine gelatin (Table 5). The viscosity of catfish skin gelatin (3.45 mPa.s) was higher than the viscosity reported for skins from Channel catfish (3.32 mPa.s) (Yang et al. 2007), 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 Hoki (10.8 mPa.s) (Mohtar et al. 2010) and lizardfish scales (7.5 mPa.s) (Wangtueai & Noomhorm 2009). Concentration of NaOH and acetic acid may have contributed to a synergistic effect on viscosity, causing complete opening up of polypeptide chain to random chain and intermolecular hydrodynamic interaction, thus leading to an increase in viscosity (Tabarestani et al. 2010). The viscosity of catfish skin gelatin is in mid range of the commercial gelatin viscosity, which is from 2.0 to 7.0 mPa.s and up to 13.0 mPa.s for specialized ones (Johnston-Banks 1990).

TEXTURE PROFILE ANALYSIS (TPA)

Table 5 compares TPA compression test results of catfish skin gelatin and bovine gelatin. The values for gumminess can be obtained from the product of hardness multiplied by cohesiveness. Similarly, chewiness is obtained from the product of hardness multiplied by cohesiveness and springiness (Wangtueai & Noomhorm 2009). Table 5 shows that there were no significant difference (p> 0.05) in gel hardness, springiness and chewiness of catfish skin gelatin and bovine gelatin. However, catfish skin gelatin showed a significantly higher (p< 0.05) cohesiveness and gumminess when compared using the same concentration of 6.67 g/100 g to bovine gelatin. The catfish skin gelatin and bovine gelatin had a springiness of 0.99 and 0.97 and a cohesiveness of 0.99 and 0.92, respectively (Table 5).

AMINO ACID COMPOSITION

The amino acid composition of catfish skin gelatin, expressed as residues per 1000 total amino acid residues, is as shown in Table 6. It was evident that glycine was abundant in catfish skin gelatin, comprising 250 residues per 1000 residues, similar to that of grey triggerfish skin gelatin (289 residues/1000 residues) (Jellaouli et al. 2011) and Nile perch skin gelatin (237 residues/1000 residues) (Muyonga et al. 2004). The imino acids (hydroxyproline and proline) of the catfish skin gelatin was moderate in content, similar to those reported on cod gelatin (Gudmundsson & Hafsteinsson 1997), which was close to 173 residues per 1000 residues. The content of imino acids, particularly that of hydroxyproline, is crucial as

TABLE 6. Amino acid composition of catfish (*Clarias gariepinus*) skin gelatin

Amino acid	Number of residues/1000
Alanine	86
Arginine	78
Aspartic acid	50
Cystine	0
Glutamic acid	85
Glycine	250
Histidine	11
Hydroxyproline	58
Isoleucine	13
Leucine	22
Lysine	33
Methionine	92
Phenylalanine	16
Proline	115
Serine	38
Threonine	25
Tryrosine	6
Valine	22
Total	1000
Hydroxyproline + Proline (Imino acids)	173

Determinations were performed in triplicate and data correspond to mean values. Standard deviations were in all cases lower than 1%

they affect the functional properties of gelatin (Gilsenan & Ross-Murphy 2000); therefore, a low amount of imino acids may indicates a poor gelling ability. According to Wangtueai and Noomhorm (2009), mammalian gelatins contained a high composition of these three amino acids especially hydroxyproline and proline, which are related to the gel-forming ability. Poppe (1997) reported that mammalian gelatins contained imino acid at approximately 30% (300 residues/1000 residues).

CONCLUSION

This study revealed the potential of catfish (*Clarias gariepinus*) skin as raw material for gelatin production, giving relatively high protein component which contributed to high viscosity and gel strength. The NaOH and acetic acid concentration along with extraction temperature was observed to significantly affect hydroxyproline yield. According to the RSM model, the optimum conditions for gelatin extraction was obtained using 0.13 mol/L NaOH and 0.09 mol/L acetic acid for 1 h as treatment, respectively and followed by a hot-water extraction at 64.92°C for 3 h. Based on the comparison with bovine gelatin, the gelatin extracted from *Clarias* catfish skin was proven to exhibit a similar quality of physicochemical properties and could be used in food industries as a replacement for mammalian gelatin.

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