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Optimization of gelatin extraction conditions from Cobia *(Rachycentron canadum)* skin and its physicochemical characteristics as compared to bovine gelatin

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Abstract

This study reported the extraction optimization and characterization of cobia *(Rachycentron canadum)* skin gelatin. Optimization study was carried out to determine the effect of CH_3COOH concentration, skin to water ratio, extraction temperature and extraction time on gelatin yield (GY) and gel strength (GS) using Response Surface Methodology (RSM). The optimum conditions were 0.15mol/L for CH_3COOH concentration, 82.4°C of extraction temperature, 6 h of extraction time and 1:6 of skin to water ratio, which produced cobia gelatin with GY of 20.10% and GS of 205.6 g. Characteristics of cobia skin gelatin (CG) were then compared to that of commercial bovine gelatin (BG). It was found that the most dominant amino acid in CG was glycine, proline and alanine. There was no difference in foaming and emulsifying properties of CG and BG at 1% concentration, but at 2% and 3% concentration, BG performed better. CG was found to have higher fat binding capacity but lower water holding capacity than BG. Least gelling concentration for CG was recorded at 2% while for BG at 1%. CG and BG had a pI at pH 6.05 and 4.82, respectively. This study shows that cobia skin gelatin has potential as halal alternative to bovine gelatin in food industry.

Introduction

Gelatin is a protein that is derived from a partial hydrolysis of collagen, which exists in the skin and bones of animals. Most commercial gelatins are currently sourced from pork skins, cow hides and cattle bones. Bovine and porcine gelatin raises ethical and religious issues among consumers. Hence, fish gelatin has been used as an alternative to mammalian gelatin to maintain the acceptability and safety of gelatin, especially for food applications. Therefore, the study of the extraction of fish gelatin has become of great interest among researchers. A number of studies on various fish species have been published from cold and warm water fish (Muyonga *et al*., 2004; Arnesen and Gildberg, 2007). Nevertheless, there are limitations in the application of fish gelatin, such as the shortage of raw material supply and its lower gelatin quality compared to mammalian gelatin. Thus, the search for a sustainable source of fish gelatin is never ending.

Cobia *(Rachycentron canadum)*, a marine fish species has been chosen as a potential candidate for gelatin extraction due to its thick skin (6% from whole cobia weight) and high gelatin yield (19%) (Amiza and Aishah, 2011). It is widely distributed in tropical and subtropical sea and extensively cage cultured in Taiwan, China and Vietnam (Yang *et al.*, 2008). Its rapid growth and excellent flesh quality have led to the increasing demand for this species, especially for the food industry, such as the sashimi or sushi market, thus indirectly generating large amounts of waste (skin, bone, scale and head) (Yang *et al*., 2008). To date, studies on cobia have mainly focused on the cobia aquaculture. Only a few studies have been reported on the utilization of cobia waste, i.e. characterisation of collagen from cobia skin (Zeng *et al*., 2012), extraction of retorted skin gelatin hydrolysate from cobia skin (Yang *et al.*, 2008), effect of drying and freezing of cobia skin on gelatin properties, as well as the effect of palm oil incorporation on the properties of biodegradable cobia skin gelatin films (Amiza and Wong, 2012). Until now, no study has been reported on the optimization of the extraction conditions of cobia skin gelatin and only a few physicochemical properties, such as chemical analysis and turbidity of cobia gelatin have been reported. Thus, this prompted the author to carry out the optimization study on cobia skin gelatin extraction and the characterisation of cobia skin gelatin compared to bovine gelatin.

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Table 1. Observed responses for optimization study

Run	Factors (actual value)				Response	
	A: Concentration of acid (mol/L)	B: Extraction temperature (°C)	C: Extraction time (hr)	D: Skin / water ratio	Gelatin Yield (%)	Gel Strength (g)
1	0.10	75.0	4.5	2.0	15.79	203.00
2	0.05	75.0	4.5	4.0	15.99	119.30
3	0.10	75.0	1.0	4.0	9.40	300.20
4	0.05	50.0	1.0	6.0	6.20	117.10
5	0.05	50.0	8.0	6.0	11.40	28.20
6	0.10	75.0	4.5	4.0	12.00	237.84
7	0.05	100.0	8.0	2.0	18.40	44.50
8	0.10	75.0	4.5	4.0	12.36	243.87
9	0.15	100.0	8.0	2.0	21.00	86.80
10	0.15	100.0	1.0	6.0	12.40	228.10
11	0.15	50.0	8.0	6.0	15.40	111.10
12	0.05	100.0	8.0	6.0	29.06	73.50
13	0.15	75.0	4.5	4.0	17.79	199.20
14	0.15	100.0	1.0	2.0	21.59	284.60
15	0.10	75.0	4.5	6.0	18.60	241.80
16	0.05	100.0	1.0	2.0	11.42	270.40
17	0.05	100.0	1.0	6.0	24.05	67.60
18	0.15	50.0	8.0	2.0	14.99	41.30
19	0.15	50.0	1.0	6.0	7.00	110.10
20	0.10	75.0	4.5	4.0	12.66	253.33
21	0.15	100.0	8.0	6.0	33.99	45.50
22	0.10	75.0	8.0	4.0	20.40	124.80
23	0.05	50.0	8.0	2.0	12.10	2.50
24	0.10	75.0	4.5	4.0	12.33	316.58
25	0.05	50.0	1.0	2.0	4.40	16.50
26	0.10	75.0	4.5	4.0	12.66	219.92
27	0.10	50.0	4.5	4.0	10.62	49.90
28	0.10	75.0	4.5	4.0	12.33	292.32
29	0.10	100.0	4.5	4.0	23.59	110.70
30	0.15	50.0	1.0	2.0	4.80	149.90

Values are the mean ± standard deviation of triplicate. a–b Means with the same superscripts within a row are significantly different ($p < 0.05$).

Material and Methods

Materials

One hundred kilogram of cage cultured marine cobia (average individual fish weight of 5 kg, 6 months old fishes, average length of 2.5 feet) were purchased from a supplier in Langkawi Island, Kedah, Malaysia. Ice storage was used during transportation of the fish to the laboratory to maintain the freshness. Cobia fish were eviscerated and filleted. Then the skin was separated from the fillet manually. Next the skin was cut into small pieces of about 1 cm^2 and washed with tap water to remove any contaminants. Then, the cleaned skin was packed in polyethylene bags and kept frozen (-40°C) until further use. All the chemicals and reagents used in this study were of analytical grade. Commercial bovine gelatin was purchased from Halagel (M) Sdn Bhd.

Optimization study

The optimization study determined the effect of CH_3COOH concentration, extraction temperature, extraction time and skin to water ratio on the gelatin yield and gel strength. Response surface methodology (RSM) was applied to optimize the extraction parameters by using a statistical package Design-Expert version 8.0.4 (Stat Ease Inc., Minneapolis, USA). A Central Composite Design (face-centred) was employed with four independent variables as shown in Table 1. The independent variables were concentration of acetic acid (A: 0.05-0.15M), extraction temperature (B: 50-100°C), extraction time (C: 1 – 8 hours), skin/water ratio (D: 1/2 - 1/6) at 3 levels (-1, 0, +1). Gelatin yield and gel strength were set as the response variables. The experimental design consists of 30 points with 16 factorial points, 8 axial points and six replicates of the centre point.

Pre-treatment and extraction of cobia skin gelatin (CG)

Gelatin extraction was carried out based on the procedure described by Zhou and Regenstein (2004). Thawed skins (40 g) were treated with acetic acid at different concentrations (factor A) for 1 hour at 4°C. The treated skin was drained using a muslin cloth and rinsed twice with tap water. Then, the samples were transferred into a conical flask and were mixed with distilled water at different total ratios of skin/water (Factor D). Aluminium foil was used to cover the flasks and samples were extracted in a shaking water bath (180 rpm) at different extraction temperatures (Factor B) and extraction times (Factor C) depending on the design. Subsequently, the gelatin solution was centrifuged at 10000 g for 30 minutes and filtered by muslin cloth. Finally, the gelatin solution was freeze dried prior to determination of the gelatin yield and gel strength.

Determination of gelatin yield and gel strength

Calculation of gelatin yield and gel strength were

carried out using the procedure by Kasankala *et al.* (2007). Gelatin yield was calculated as the ratio of weight of dried gelatin to the total weight of fish skin on a wet basis.

Statistical analysis

The optimization data obtained were statistically analysed by Design-Expert 6.0.11, (State-Ease, Inc., Minneapolis MN, USA). The significance of all terms in the polynomial were analysed statistically at $p<0.05$. Verification experiments were conducted in three replicates under optimal conditions to compare the predicted values and actual values of the responses.

Characterisation of cobia skin gelatin (CG)

CG extracted at optimum condition obtained from optimization study was freeze-dried and characterised in terms of its physicochemical properties. The characteristics of commercial BG were also determined as a comparison.

Chemical composition

Determination of chemical analysis on CG and commercial bovine gelatin (BG) were determined according to the methods of the Association of Official Analytical Chemists (AOAC 2000).

Determination of amino acid composition

Amino acid compositions were determined as described by Zarai *et al.* (2012) using an amino acid analyser. The compositions of the amino acids were reported as a percentage.

Determination of foaming properties

The foam properties including foaming expansion (FE) and foaming stability (FS) of the gelatin solutions were determined as described by Shahidi *et al*. (1995).

Determination of emulsifying properties

The emulsion activity index (EAI) and emulsion stability index (ESI) of the gelatin samples were determined according to the method of Pearce and Kinsella (1978).

Determination of water holding capacity (WHC)

The water-holding capacity was measured by following the procedure of Lin *et al*. (1974) .

Determination of fat binding capacity (FBC)

The fat-binding capacity was measured using a modified method of Lin *et al*. (1974).

Determination of viscosity

The gelatin solutions were made by dissolving lyophilized gelatin powder in distilled water (6.67% (w/v) and heating to 60°C for 30 minutes. To determine the viscosity, the Brookfield DV-III Viscometer was used. In this analysis, a small sample adapter and no. 1 spindle at 100rpm were used. The viscosity during the cooling of the gelatin solution from 40°C to 5°C was measured at 0.2°C/min (Arnesen and Gildberg, 2007).

Determination of least gelation concentration (LGC)

Determination of least gelation concentration was employed as described by Coffman and Garcia (1977). Appropriate gelatin suspensions of 0.7 to 2.5% (w/v) were prepared with 5 ml distilled water in test tubes. The test tubes containing these suspensions were then heated for 1h at 80°C in a water bath, followed by rapid cooling under running cold water. The test tubes were further cooled to 4°C for 2 h in a chiller. The least gelation concentration was determined as the concentration when the sample from the inverted tube did not fall down or slip.

Determination of isoelectric point (pI)

Isoelectric points was determined as described by Ahmad and Benjakul (2011). The gelatin samples were dissolved in distilled water at a concentration of 0.5 mg/ml. The mixture was stirred at room temperature for 6 h. The z-potential of each sample (20 ml) was measured using a zeta potential analyser (ZetaPALS, Brookhaven Instruments Co., Holtsville, NY, USA). The Z-potentials of the samples were adjusted to different pH levels with 1.0M nitric acid or 1.0M KOH using an autotitrator (BI-ZTU, Brookhaven Instruments Co., Holtsville, New York, USA). The pI was estimated from the pH rendering a z-potential of zero.

Statistical analysis

All experiments were performed in triplicate. Data were presented as mean±standard deviation and the probability value of less than 0.05 was considered significant. The independent t-test was performed to determine the significant difference between the characteristics of BG and CG. Analysis was performed using SPSS software (SPSS 11.5 for Windows, SPSS Inc, Chicago, IL, USA).

Results and Discussion

Optimization study

The experimental conditions and the responses of optimization study are shown in Table 1. Based on the results obtained, it was found that the gelatin yield and gel strength ranged from 4.4% to 33.9% and 0.0 g to 316.58 g, respectively. Gelatin yield was

Table 2. ANOVA for the regression model and the respective model terms for optimization

Source	Sum of Squares	df	Mean Square	F Value	Prob > F
Gelatin Yield (GY) (%)					
Model	1.03	3	0.34	38.38	<0.0001
B	0.62	1	0.62	68.82	<0.0001
C	0.37	1	0.37	41.72	<0.0001
D	0.041	1	0.041	4.59	0.0417
Residual	0.23	26	8.950E-003		
Lack of Fit	0.23	21	0.011	146.25	<0.0001
Pure error	3.782E-004	5	7.564E-005		
Total	1.26	29			
Gel Strength(GS) (g)					
Model	481.99	6	80.33	19.55	< 0.0001
A	47.51	1	47.51	11.56	0.0025
B	49.75	1	49.75	12.11	0.0020
C	113.75	1	113.75	27.68	< 0.0001
BD	34.54	1	34.54	8.41	0.0081
B^2	235.76	1	235.76	57.38	< 0.0001
Residual	94.50	23	4.11		
Lack of Fit	88.30	18	4.91	3.96	0.0671
Pure error	6.20	5	1.24		
Total	576.49	29			

df: degree of freedom, A: concentration of acetic acid, B: extraction time, C: extraction temperature, D: skin to water ratio

calculated as the ratio of weight of dried gelatin to the total weight of fish skin on a wet basis.

Model fitting

Based on the results, the software suggested a linear model for gelatin yield and a quadratic model for gel strength. After removing the insignificant terms at $P < 0.05$ confidence levels, the mathematical model representing the GY and GS as a function of the independent variables within the region was expressed by the following equation:

$$\text{Gelatin yield} = 1.14 - 0.18^{*}B + 0.14^{*}C + 0.048^{*}D$$

$$\text{Gel strength} = 15 + 1.62^{*}A + 1.66^{*}B - 2.51^{*}C + 0.19^{*}D - 1.47^{*}B^{*}D - 5.72^{*}B^{2}$$

where A is the acetic acid concentration, B is the extraction temperature, C is the extraction time and D is the skin to water ratio.

The linear model suggested for the gelatin yield of CG was not in agreement with previous studies that have reported on the optimization of gelatin from grass carp skin (Kasankala *et al*., 2007) and rainbow trout skin (Tabarestani *et al*., 2010), which found that the predicted model for gelatin yield was a quadratic model.

However, the quadratic model suggested for the gel strength of CG was consistent with previous studies conducted on grass carp skin (Kasankala *et al*,. 2007) and rainbow trout skin (Tabarestani *et al.,* 2010). Meanwhile, both linear and quadratic models could be used to predict the gel strength model for gelatin from lizard fish scales and Alaskan Pollock skin (Zhou and Regenstein, 2005; Wangtueai and Noomhorm, 2009). The difference in the prediction model for gelatin yield and gel strength could be due to the differences in raw materials, pre-treatment and extraction condition used.

Analysis of variance (ANOVA)

Table 2 shows the ANOVA results obtained after model reduction was performed. For gelatin yield, the model F-value was 38.38 and the p-value was <0.0001, which indicated that the model was significant. In addition, the significant lack of fit showed that the model was not capable of estimating all the possible information for the model independently and that it was not a good response predictor. However, the "Predicted R-Squared (Pred R^2)" of 0.7317 and "Adjusted R-Squared (adj-R^2)" of 0.7945, which did not differ much with each other, as well as the Adeq Precision being more than 4 (21.815), indicated an adequate signal that showed that this model could still be used to navigate the design space.

The F-value of the model for gel strength was 19.55 and the p-value was <0.0001, which implied that the model was significant. The Insignificant Lack of Fit value implied that the model fits well with the experimental data. The "Predicted R-Squared

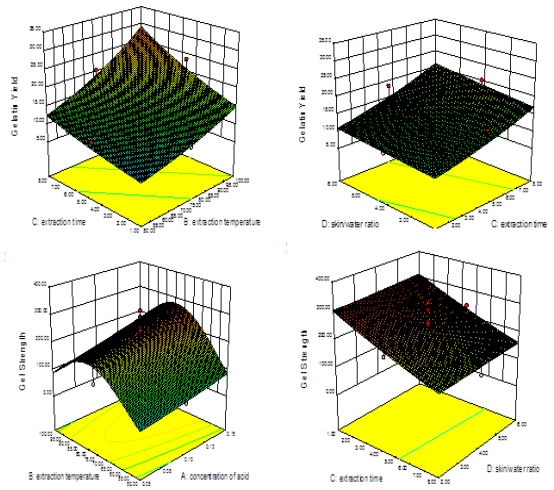


Figure 1. Response surface plots of interactive effects of experimental factors on gelatin yield and gel strength

(Pred R^2)" 0.6743 was in reasonable agreement with the "Adjusted R-Squared (adj-R^2)" of 0.7933, which signified that non-significant terms have not been included in the model. The Adeq Precision measures the signal to noise ratio, in which a ratio greater than 4 is desirable. The ratio of 16.035 for gel strength indicated an adequate signal, which showed that this model could be used to navigate the design space.

Response surface plots

The relationships between the responses and interactive effects of the experimental variables are depicted in Figure 1. The extraction temperature had a linear effect on the gelatin yield. A similar result was obtained by Al-Saidi *et al*. (2011) who reported that the higher the temperature used, the higher the gelatin yield for all acetic acid concentrations. Figure 1 also shows that the gelatin yields were also affected by the extraction time whereby the yield gradually increased with the increase in extraction time. The skin to water ratio also had a linear effect on the gelatin yield. The gelatin yield and quality were improved by increasing the amount of water during extraction since a high ratio of water to collagen-containing material assists in removing gelatin from the surface of the pre-treated skins (Nasrallah *et al*., 1993).

For gel strength (GS), the results showed that the concentration of acetic acid (A) linearly affects GS, which was consistent with the findings of Cho *et al*. (2006). Figure 1 also indicated that the GS also increased with the increase in extraction temperature and decreased as the temperature increased above 80ºC. However, Yang *et al*. (2007) reported that the GS of channel catfish gelatin decreased at lower temperature i.e. after 60◦C. This shows that cobia gave higher optimum extraction temperature compared to channel catfish. This differences could be due to differences in the raw material i.e. cross linking of collagen in fish skin from different fish species (Kolodziejska *et al.*, 2008). Another factor that significantly affected GS was the extraction time. The shorter the extraction time, the higher the GS obtained. The GS declined at higher extraction time and temperature due to the breakage of hydrogen bonds and free amino acid hydroxyl groups (Cho *et al*., 2006). In addition, high extraction temperature also caused protein degradation and denaturation that produced small proteins, which had a lower gel strength value (Yang *et al.,* 2007).

Optimization and verification

The optimization process was carried out to determine the optimum conditions for gelatin extraction. The desired goals for experimental factors were set within the range while for the responses the gelatin yield (GY) and gel strength (GS) were set to the maximum values. Under the optimum conditions, i.e. concentration of acetic acid 0.15 mol/L, extraction temperature 82.4ºC, extraction time 6 hours and skin to water ratio 1:6, the predicted values were 20.59% of GY and 226.86 g of GS. Using the same optimum conditions, verification experiments were conducted and the experimental values obtained were GY of 20.10% and GS of 205.6 g, which shows no significant different with the predicted response values using one sample t-test.

Gelatin yield

GY was calculated as the ratio of weight of dried gelatin to the total weight of fish skin on a wet basis. Therefore, a large range of yield values were found for various types of gelatin as the water content may vary due to the different types of treatment of the skin (freezing, salting, scraping, washing and draining) (Arnesen and Gildberg, 2007). The range of gelatin yield obtained from the optimization data was between 4.4 to 33.9%. The GY from cobia skin was higher compared to that reported for other species, such as skins of channel catfish (19.2%) (Yang *et al.*, 2007), grass carp (19.83%) (Kasankala *et al*., 2007) and unicorn leatherjacket (6.12-11.54%) (Ahmad and Benjakul, 2011). However, a higher GY was reported for salmon (39.7%) and cod (44.8%) (Arnesen and Gildberg, 2007).

Gel strength

GS is one of the important properties that determine the quality of gelatin. Typically, fish gelatin has a GS value ranging from 0 to 426 g, compared to 200–300 g for bovine or porcine gelatin (Karim

Table 3. Amino acid composition of gelatin from cobia skin and bovine

Amino Acid (g/100g)	Cobia skin gelatin	Bovine gelatin
Non polar Hydrophobic		
Alanine (Ala)	8.80	8.41
Valine (Val)	2.33	2.07
Leucine (Leu)	2.10	1.89
Isoleucine (Ile)	1.14	1.01
Phenylalanine (Phe)	1.81	1.60
Methionine (Met)	1.84	0.22
Proline (Pro)	10.08	12.66
Tryptophan	ND	0.48
Polar uncharged		
Glycine (Gly)	20.98	37.05
Serine (Ser)	3.26	2.93
Threonine (Thr)	2.45	0.82
Tyrosine (Tyr)	0.64	1.16
Cysteine (Cys)	0.0	0.47
Polar Acidic		
Aspartic acid (Asp)	4.86	3.29
Glutamic acid (Glu)	8.30	5.43
Polar basic		
Lysine (Lys)	3.29	4.86
Arginine (Arg)	7.63	5.09
Histidine (His)	ND	ND
Amino acid derivatives		
Hydroxyproline (Hyp)	7.14	10.67
Hydroxylysine (Hyl)	ND	ND

and Bhat, 2009). The range of GS obtained from optimization data was between 0.0 g to 316.58 g, which was similar to that reported for grass carp skin (267 g) (Kasankala *et al.*, 2007), channel catfish skin (252 g) (Yang *et al*., 2007), catfish skin (278.72 g) (See *et al.*, 2010) and snakehead skin (311.18 g) (See *et al*., 2010). However, the GS values obtained in this study were higher than commercial gelatin from cod (71 g) and salmon (108 g) (Arnesen and Gildberg, 2007) and cold water fish (3.91 g) (See *et al*., 2010), but lower than those of Pangasius catfish (324.53g) and red tilapia (487.61 g) (See *et al*., 2010). The source and type of collagen of raw material influence the properties of the resulting gelatin. Apart from the origin of the raw material, the methods and conditions applied during the processing of fish gelatin also affect the GS (Karim and Bhat, 2009).

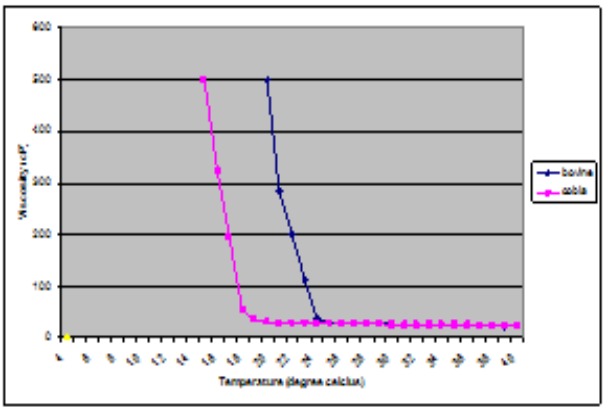
The GS of commercial gelatin ranged from 100 to 300g (Karim and Bhat, 2009). For fish gelatin, a Bloom value between 150 to 300 g was found to be suitable for use in the microencapsulation of food flavours such as vegetable oil, lemon oil, garlic flavour, apple flavour, and black pepper (Soper, 1999). In addition, fish skin gelatin may also be used in the production of soft gelatin capsules, which require gelatin with GS 150 to 200 g. Therefore, the value of GS for cobia skin gelatin suggested that the properties of gelatin in this study were promising for use for certain applications to replace mammalian gelatin.

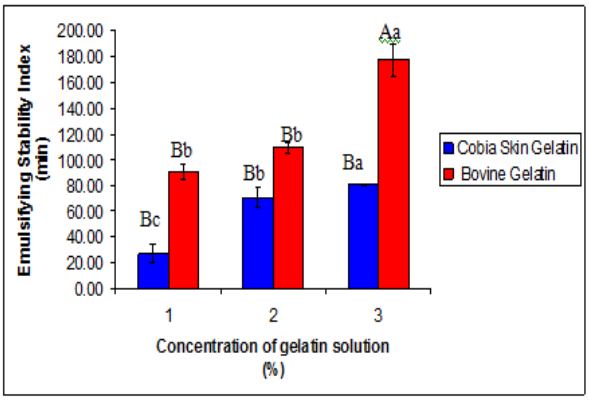
Characterisation of cobia skin gelatin

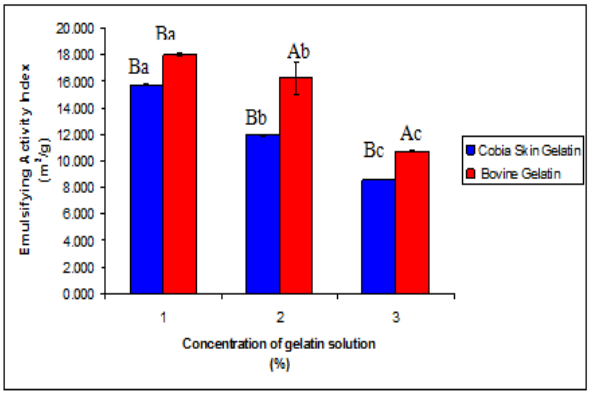
Viscosity

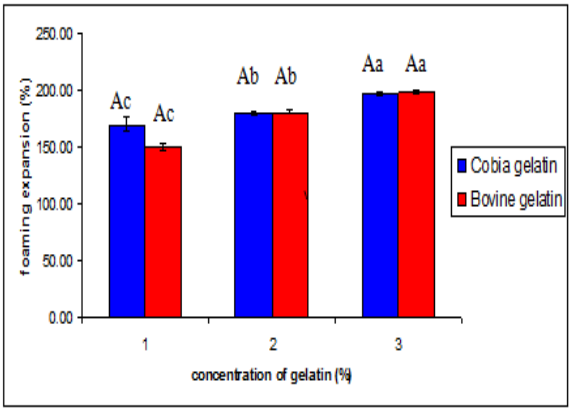
The viscosity of CG and BG from 40°C to 4°C are depicted in Figure 2(a). Based on the results, it can be seen that above 15oC, both gelatin solutions had equal viscosities (ranging from 23-500 cP). Overall, the viscosity of gelatin solutions increased slowly with decreasing temperature, however as the temperature approached the gelling point, the viscosity increased drastically. There was a similar trend in the viscosity of both gelatin samples with temperature. Both gelatins exhibited the same maximum value of viscosity, which was 500 cP. The viscosity of gelatin solutions has a close relation with the molecular weight (MW) and polydispersity whereby a higher MW will increase the viscosity (See *et al*., 2010).

BG showed a higher gelling point (20°C) compared to CG (15°C). The results were in agreement with Cho *et al*. (2005) who reported that yellowfin tuna skin gelatin had a slightly lower gelling temperature compared to mammalian gelatin.









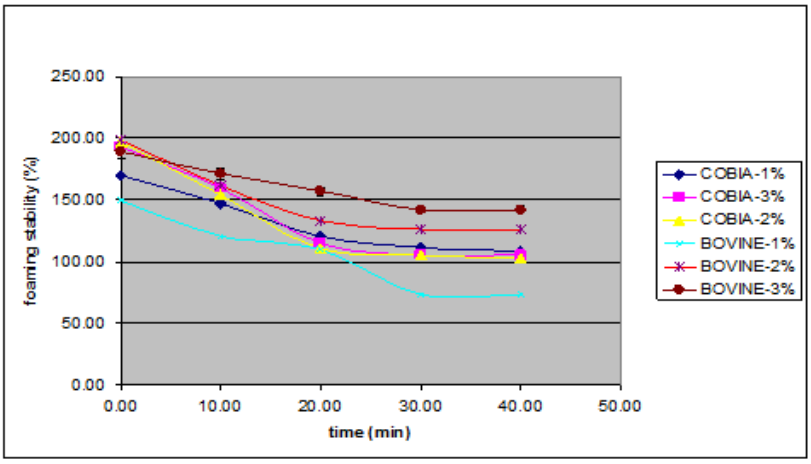


Figure 2(a). Viscosity of cobia skin gelatin (CG) and commercial bovine gelatin (BG) at different temperature. Figure 2(b) - 2(e): Emulsifying activity index (EAI), emulsifying stability index (ESI), foaming expansion and foaming stability of CG and BG at different concentrations. Different small letters (a-c) indicate significant differences between different concentration for each gelatin source ($p< 0.05$). Similar capital letters (A) indicate no significant difference between the gelatin source within the same gelatin concentration ($p < 0.05$)

However, CG had a higher gelling temperature than that reported for cold water fish, as the gelling point is influenced by the content of imino acid (Karim and Bhat, 2009). The low gelling temperature of CG could be useful for certain applications, such as in the manufacturing of capsules, stabilizing the foam upon cooling in marshmallows (Karim and Bhat, 2009) and flavour release for cold desserts (Gómez-Guillén *et al*., 2011).

Chemical composition

The chemical composition of CG (in percentage) were 7.01±0.75 moisture, 89.7±0.17 crude protein, 0.71±0.05 ash and 2.58±0.19 crude fat. As for commercial BG, it is consisted of 12.52±0.17 moisture, 84.72±5.74 crude protein, 0.75±0.09 ash

and 2.01±0.34 crude fat. The differences in moisture content could be due variation in the drying conditions and the absolute ambient humidity. British Standard Institution (BSI 1975) regulates the maximum moisture content for gelatin at 14%.

CG consisted of 89.7% of crude protein, which is slightly higher than that of bovine (84.72%). This result was in similar range with the previous findings for gelatin from skate skin (92.31%) (Cho *et al.*, 2006). CG had a high percentage of crude protein, which was probably due to high protein content of the collagenous material in the fish skin itself.

The ash content for CG (0.71%) was slightly lower than for BG. The value, however, could be considered as low and fulfilled the standard regulation as ash content up to 2% is acceptable for food applications (Kasankala *et al*., 2007). The ash content varies over a wide range because of the type and mineral content of raw material and the extraction methods. Therefore, it can be concluded that the low percentage of ash indicated an efficient extraction process.

The fat content in CG extracted in this study (2.58%) was higher compared to BG (2.01%) and the gelatin from other fish species, such as Catfish and Nile tilapia (Songchotikunpan *et al*., 2008) which were in the range of 0.3-1.17%. The differences in fat content could be affected by fish species in that they contain different amounts of fat in the skin, and whether or not defatting was carried out on the fish skin.

Amino acid composition

The amino acid composition of CG is shown in Table 3. Total amino acid content in CG and BG is 86.65% and 99.51%, respectively. Compared to protein content using Kjeldahl method, total amino acid was is lower for CG, but higher for BG. Ratnasari *et al.* (2013) whom reported the total amino acid composition of for skin gelatin from fresh water fishes (Pangas catfish, Asian redtail catfish, Nile tilapia, striped snakehead, commercial fish gelatin) reported a lower range of 65.5-75.4%. The difference in total amino acid among gelatin samples depends on gelatin content in the sample and loss of amino acid via peptide bond cleavage and amino acid degradation during acid hydrolysis (Darragh *et al*., 1996).

The most dominant amino acid in CG is Glycine (Gly), which was about 20.98% of total amino acids. Besides Gly, other important amino acids are Proline (Pro) and Hydroxyproline (Hyp), which were 10.08% and 7.14%, respectively. Similar findings were reported for cobia skin collagen, which is rich in Gly, Alanine (Ala), Pro and Hyp (Zheng *et al.*, 2012). The amino acid compositions of collagen and gelatin are very similar to that of the parent proteins (Gómez-Guillén *et al*., 2010). The results obtained were considered high and comparable to those reported for Grass carp (19.6% Gly, 2% Pro and 11.27% Hyp) (Kasankala *et al.*, 2007) and Nile tilapia (21.18% Gly, 8.83% Pro, 8.70% Hyp) (Songchotikunpan *et al.*, 2008). A high percentage of Gly affects the water binding properties of gelatin and leads to the high viscosity, gel strength and melting point of gelatin (Pranoto *et al*., 2007).

Besides Gly, Pro and Hyp, hydrophobic amino acids (Ala, Val, Leu, Iso, Leu, Pro, Phe and Met) are also considered to contribute to the high Bloom value (Badii and Howell, 2006). The amounts of these amino acids in CG, as shown in Table 3, were in agreement with previous studies. Cysteine (Cys), Histidine (His), Hyp and Try were not detected in this gelatin. Try and Cys are usually absent in collagens and gelatins (Karim and Bhat, 2009).

Different fish species showed different amounts of amino acid, as these vary with the source of collagen (i.e. the composition of gelatin is similar to the mother collagen from which it has been prepared) and also the method used for the pre-treatment of raw materials (i.e. acidic pre-treatment) commonly resulted in a higher amount of acidic residue since some of the glutamine and asparagine residues might have converted or oxidized into their acidic forms (Giménez *et al*., 2005; Zarai *et al*., 2012). In addition, the amounts of the amino acids also depend on the environmental temperature of the fish habitat (Karim and Bhat 2009).

Table 3 shows that for all types of amino acid except Gly, Pro, Hyp, Tyr and Lys; their amount is higher in CG compared to BG. According to Karim and Bhat (2008) the main differences between fish and mammalian gelatins are the different contents of the imino acids, Pro and Hyp, which stabilize the ordered conformation when gelatin forms a gel network. The higher Hyp content in BG is associated with the formation of hydrogen bonds between water molecules and free hydroxyl groups of amino acids in gelatin, which are essential for gel strength (Arnesen and Gildberg, 2007).

Emulsifying properties

The emulsifying activity index (EAI), a measurement of the area of interface stabilised per unit weight of protein (m2/g), relates to the ability of a protein to coat an interface (Pearce and Kinsella, 1978). The results showed that the emulsifying

activity index decreased as the concentration of gelatin solution increased, regardless of the source of the gelatin (Figure 2(b)). The EAI for commercial BG and CG at 1% concentration was significantly higher compared to other concentrations (2% and 3%). Similar results were also reported by Ahmad and Benjakul (2011) and Nagarajan *et al*. (2012). A low protein concentration favours higher EAI due to the ability of the protein to diffuse and adsorb at the oil-water interface in a controlled manner whereas at high protein concentrations, the activation energy barrier does not allow protein migration to take place in a diffusion-dependent manner, thus limiting the diffusion and leading to an accumulation of proteins in the aqueous phase.

At higher concentrations (2% and 3%), the EAI of CG was significantly lower than that of BG. These results were in reasonable agreement with that reported by Aewsiri *et al.* (2009) for cuttlefish skin (18.17-24.30 m^2/g) compared to bovine (28.27 m^2/g). EAI for CG obtained in this study (8.527 -15.709 m2/g) were lower than those reported for gelatin from unicorn leatherjacket skin (13.49-37.48 m^2/g) and marine snails (32.77 m^2/g) (Ahmad and Benjakul, 2011; Zarai *et al*., 2012). This possibly resulted from the difference in the intrinsic properties, amino acid composition and conformation of proteins between the two sources of gelatin.

The emulsion stability index (ESI) of gelatin extracted from cobia skin and bovine were also evaluated in this study at three concentrations and are shown in Figure 2(c). ESI is a measurement of the ability of the protein solution to maintain a stable emulsion over a period of time by preventing the flocculation and coalescence of the oil globules (Zayas, 1997). It was found that for both types of gelatin, increasing the concentration from 1% to 3% of gelatin solution increased the emulsifying stability index, except for BG, for which no significant difference was found between the concentrations of 1% and 2%. The highest ESI was found at 3% concentration for CG (80.77±0.38) and BG (177.38±12.05) ($p < 0.05$). The results obtained were in a good agreement with Ahmad and Benjakul (2011). Based on the results, ESI was found to be strongly dependent on the gelatin concentration, which affected the droplet size and the viscosity of the solution. According to Li and Xia (2011), an increase in the concentration of the solution promoted the fragmentation of oil into smaller droplets and the aqueous phases were increasingly viscous. These reduced the droplet diffusion in the solution, facilitated more protein/oil adsorption at interfaces and stabilized the contact between droplets, thus increasing the stability towards emulsion collapse (Li and Xia, 2011).

It was also observed that BG exhibited higher ESI than CG at the higher concentration (3%), whereas at the lower concentrations (1% and 2%), no significant difference was observed. A higher ESI for BG (31.23 ± 0.90) was also reported by Aewsiri *et al.* (2009) in their study on cuttlefish skin gelatin. This can be explained by the hypothesis that at higher concentrations, these two types of gelatin may have different structural properties, which may contribute to different values of ESI. Longer peptides are able to form the stronger and stiffer films surrounding the oil droplets, thereby increasing the stability towards emulsion collapse (Nagarajan *et al.*, 2012). Surh *et al*. (2006) also reported that the oil-in-water emulsion prepared with high molecular weight gelatin was more stable than that prepared with low molecular weight gelatin.

Foaming properties

Figures 2(d) and 2(e) show the foaming expansion (FE) and foaming stability (FS) of CG and BG at different concentrations. Figure 2(d) depicts that the FE of gelatin increased with increasing gelatin concentrations ($p < 0.05$), regardless of the source of the gelatin. A similar trend was reported for unicorn leatherjacket skin gelatin (Ahmad and Benjakul, 2011) and cuttlefish skin gelatin (Balti *et al*., 2011). However, there was no significant difference between cobia and BG for all concentrations ($p<0.05$).

The FE for CG was higher than those reported for marine snail gelatin, 75.44% (Zarai *et al*. 2012) and smooth hound skin gelatin, 103-134.52% (Bougatef *et al.*, 2012). The difference in foaming properties between these types of gelatin could possibly be due to the molecular weight distribution of gelatin. CG with a lower molecular weight of protein compared to bovine could migrate to the air-water interface more effectively, unfolding and rearranging at the interface to express good foaming ability (Ahmad and Benjakul, 2011).

FE after whipping was monitored every 10 minutes for 40 minutes to evaluate the foam stability of gelatin. For both types of gelatin, the foam stability (FS) increased with increasing gelatin concentrations from 1% to 3% and then remained nearly constant after 30 minutes (Figure 2(e)). The same trend was reported by Balti *et al*. (2011) for gelatin from the skin of cuttlefish. An increase in the foam stability with an increasing protein concentration has been reported as a result of the formation of stiffer foams (Lawal, 2004). At higher concentrations of gelatin solution, foams were denser and became more

stable because of an increase in the thickness of the interfacial films, which led to the FS value becoming constant (Zayas, 1997).

At the lower concentration of gelatin solution (1%), the foam stability for CG was higher than that for BG. However, as the concentration increased from 2% to 3%, the BG showed slightly higher foam stability compared to CG. The difference in the FS of the types of gelatin was mainly because of the difference in the hydrophobic amino acid content among the species. An increase in the hydrophobic chain and the number of attached alkyl chains per molecule of gelatin leads to a decrease in the size of emulsion droplets, thus producing more stable foam (Jeya Shakila *et al.*, 2012). In addition, the molecular weight distribution of gelatin does affect the FS. The adsorption to the air-water interface is quicker in gelatin with low molecular weight (small size of peptides), which increases the FS (Martin *et al*., 2002)

Water holding capacity (WHC) and fat binding capacity (FBC)

The results indicated that the WHC for CG (97.03±1.53) was significantly lower than for BG (160.53±7.18). The value of WHC for CG was slightly lower than that reported for gelatin from cuttlefish skin (150-200%) (Balti *et al.*, 2011) and marine snails (120%) (Zarai *et al*., 2012). The same trend was reported for cuttlefish skin gelatin (Balti *et al*., 2011). The low value of WHC for CG was due to the lower amounts of hydrophilic amino acids content (Jeya Shakila *et al.*, 2012), as discussed previously. In addition, the WHC may also be affected by the microstructure, specifically the primary chemical structure of the gelatin (Zarai *et al*., 2012). Therefore, the lower WHC value obtained for CG can be explained by the existence of fewer pores and voids in its structure compared to BG, which reduced its ability to retain water against a gravitational force. Another reason for the different WHC value between these two gelatins is the particle size (Zarai *et al*., 2012). BG, which was commercially prepared to a fine powder, increased the surface tension of water, thus making it able to hold more water compared to CG with larger particles (Zarai *et al*., 2012).

In contrary to WHC, the opposite trend was observed for the fat binding capacity (FBC) between cobia skin and BG. CG had a higher fat binding capacity (164.01) compared to commercial BG (105.66). The possible explanation for the differences in FBC was the degree of exposure of the hydrophobic amino acid (Balti *et al*., 2011). As shown in Table 3, the hydrophobic amino acids of CG were higher than those from BG, which corresponded to a higher fat binding capacity. The high fat binding capacity indicated that the CG has potential and could be a useful additive in food formulation such as for processing of dough during the manufacturing of low-fat cookies and cakes.

Least gelation concentration (LGC)

In this study, LGC was used as an indicator for gelation. LGC is defined as the lowest protein concentration at which gel remains in the inverted tube (Coffman and Garcia, 1977). The determination of LGC was carried out at 0.7% to 2.5% for both CG and BG. BG formed a gel at a very low concentration of 1% whereas the LGC for CG was at 2%. The results obtained showed that the LGC for BG is lower than for CG. The LGC for CG is higher compared to silver carp skin gelatin (0.8- 0.95%) (Zhang *et al*., 2012). The lower the LGC of a gelatin, the better its gelling ability. Overall, both types of gelatin may act as good gelling agents.

Isoelectric point

Zeta-potential analysis revealed that CG had an isoelectric point (pI) at pH 6.05 whilst BG had a pI at pH 4.82. This is consistent with the findings on gelatin from skate skin (Cho *et al.*, 2006) and unicorn leatherjacket skin (Ahmad and Benjakul 2011), which exhibited a pI at pH range 6.45-7.26. Gudmundsson and Hafsteinsson (1997) also reported a similar pI for cod gelatin, which was 6.2 to 7.1. The relatively high isoelectric point of Type A gelatin makes it more suitable for creating oil-in-water emulsions that have a positive charge over a wider range of pH values than conventional protein emulsifiers, such as soy, casein or whey proteins (Dickinson and Lopez, 2001).

Conclusion

The present study revealed that the optimization model generated can be used to optimize cobia skin gelatin extraction with reasonable yield and gel strength. The physicochemical characteristics of cobia skin gelatin shows good potential to be used as a halal alternative to mammalian gelatin in food and pharmaceutical industry.

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