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ORIGINAL ARTICLE

Response surface methodology (RSM) for optimization of gelatin extraction from pangasius fish skin and its utilization for hard capsules

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Abstract This study was aimed to investigate the use of the response surface methodology (RSM) to improve the extraction process of pangasius *Pangasianodon hypopthalmus* fish skin gelatin. RSM was conducted based on central composite design (CCD) consisting of the two factors, namely, citric acid concentration and extraction time. The yield, moisture content, ash content, pH, viscosity, and setting point were all positively correlated with citric acid concentration, whereas the extraction duration influenced the yield, gel strength, moisture content, and setting point. The combination of these two factors improved the physicochemical properties of the final gelatin preparation. The optimized extraction process was found at the citric acid concentration of 0.3 % and the extraction time of 8 h, producing the gelatin of the quality within the standard GMIA. The gelatin resulted from the optimized processing condition was used as a raw material for hard capsules. The characteristics of the capsules produced were in accordance with those of the commercially available hard capsules.

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1. Introduction

Gelatin is the result of thermal denaturation of collagen from the animal skins, connective tissues, and bones (Nurilmala et al., 2021). The amount of gelatin production worldwide tends to increase every year. Commercial gelatin is mostly derived from the bones and skins of terrestrial animals such as porcine and bovine sources. Bovine gelatin could be contaminated with the pathogens causing diseases such as *mad cow disease*, *foot and mouth disease* and *Bovine Spongiform Encephalopathy* (BSE) (Mad-Ali et al., 2017). In addition, there are limitations of the use of porcine and bovine materials for gelatin from the religious reasons. Muslims are prohibited from eating pork, while Hindus are prohibited from eating beef (Rezaei and Motamedzadegan, 2012).

Gelatin from fish can be an alternative to meet the increasing demand for the halal gelatin. Fishery by-products such as skin can be a source for gelatin (Nurilmala et al., 2020a; Nurilmala et al., 2021). Pangasius (*Pangasianodon hypopthalmus*) skin is a potential source for sustainable production of gelatin. Pangasius is a freshwater fish having a high commercial value and the prospect of development in the community. Production of the gelatin from pangasius skin has been reported recently (Mahmoodani et al., 2014, Barmon et al., 2020, Nurilmala et al., 2021). However, optimization of the extraction process is still required (Nurilmala et al., 2021).

The quality of gelatin depends on the physicochemical properties which are strongly influenced by the origin of the raw material and the processing method. Fish gelatin is generally less stable and has poorer gelling properties than those from terrestrial animals limiting the application (Mahmoodani et al., 2014). The quality of gelatin is determined by the length of the polypeptide chains. Longer chains with higher molecular weights will produce gelatin with better functional properties and higher molecular weight. Extraction conditions that affect the quality of gelatin include the method of preparation (pretreatment), extraction temperature and time, and the intrinsic properties of collagen. The alkaline pretreatment favors the removal of non-collagenous proteins and lipids (Uranga et al., 2021).

Improving the quality of extracted gelatin can be maximized using *response surface methodology* (RSM), which is a collection of combined mathematical and statistical techniques that help evaluating processing factors to build a variable model with optimized conditions, and then describe the response with the highest yield (Fan et al., 2017). Differences in the concentration of citric acid and extraction time need to be examined to obtain the optimal conditions for extracting gelatin from pangasius fish skin. It has been reported the optimized extraction conditions of acid concentration and extraction time showed gelatin with the best quality (Mahmoodani et al., 2014).

On the other hand, capsules are used as a shield for drugs, protecting them from environmental influences and help preventing oxidation of the contents of the capsules (Yang et al., 2020). There are two types of capsules, namely hard and soft ones. The hard capsules have a cylindrical shape and consisting of the two parts. The part of short cylindrical shape is called the cap, while the long cylindrical shape is called the body. Whereas, soft capsules consist of only one part. They are usually used for medicines that need to be dissolved in oil or other liquids to help the drug being absorbed in the stomach (Deepak et al., 2014). Capsules that are widely used are hard capsules because they can be used for both solid and liquid drugs (Fauzi et al., 2020).

The objective of this study was to produce gelatin from the pangasius skin. RSM was conducted to determine the optimized processing conditions. Then, the resulted gelatin was applied to the production of hard capsules, and further the physiochemical properties of the capsules obtained were compared with the commercially available ones.

2. Materials and methods

2.1. Skin preparation

The skin from the specimens of fresh pangasius (*Pangasianodon hypopthalmus*) was cleaned from waste materials such as meat scraps and fat, using a sharp knife. The skin was then washed intensively with tap water. The washed skin was then cut with to a size of about 1 × 1 cm and washed again with tap water as above. The cleaned skin thus prepared was stored in polyethylene bags at −18 °C for until use.

2.2. Gelatin preparation

As a pretreatment, the skin was treated with NaOH, then with citric acid as follows. In the first treatment, the skin was immersed in 0.05 M NaOH solution with a ratio of the skin and the solvent 1:4 (w/v) for 1 h (Nurilmala et al., 2021) followed by washing with tap water. The second treatment was immersion in citric acid with different concentrations (w/v) according to the formula obtained from the running software with an upper limit of 0.3%, the lower limit of 0.1%, with the midpoint of 0.2%. The pretreatment process was carried out for 12 h, and then washed with distilled water until the pH became around 7. The gelatin was extracted with distilled water from the treated skin at 65 °C for different extraction time according to the running software formula with the lower limit of 6 h, the upper limit of 8 h, with the midpoint of 7 h. The ratio of the skin and distilled water for the extraction was 1:1. The treated gelatin was filtered through calico cloth and cotton to produce liquid gelatin, which was then dried using an oven at 50 °C for 24 h. The resulting gelatin was analyzed for the yield, air content, ash content, pH, gel strength, and viscosity.

2.3. Experimental design

Experimental design of gelatin extraction from the pangasius fish skin was carried out by using RSM based on Central Composite Design (CCD). RSM was used to produce product formulations towards the external production factors such as citric acid concentration (X1) and extraction time (X2) as the independent variables. Applications for making a gelatin trial design was done by using the *Design Expert software* version 10. The independent variables and treatment codes can be seen in Table 1.

The running software resulted in 13 treatments with the independent variables of citric acid concentration and extraction time. The response variables in this study were yield, gel strength, moisture, ash content, pH, viscosity and setting point. The experimental design can be seen in Table 2.

2.4. Preparation of hard capsules

The hard capsules were made from the obtained gelatin. Solid gelatin was mixed with distilled water at the ratio of 1:2 (w/w), then dissolved in a melter at around 85–90 °C. The dissolved gelatin was printed on a capsule mold plate at around 50–60 °C. The pin bar that would be used to be printed on

Table 1 Determination of the independent variables (acid concentration and extraction time).

Variables	Code	Range and level				
		-α (-1.414)	−1	0	1	α (1.414)
Acid concentration (%)	X1	0.0585	0.1	0.2	0.3	0.3414
Extraction time (h)	X2	5.58	6	7	8	8.41

Table 2 Experimental design for the production of fish skin gelatin.

Treatment	Factor codes		Actual factors	
	X1	X2	Citric acid concentration (%)	Extraction time (h)
1	α (1.414)	0	0.3414	7
2	0	0	0.2	7
3	0	0	0.2	7
4	0	-α (-1.414)	0.2	5.58
5	-α (-1.414)	0	0.0585	7
6	1	1	0.3	8
7	0	α (1.414)	0.2	8.41
8	1	−1	0.3	6
9	−1	1	0.1	8
10	0	0	0.2	7
11	0	0	0.2	7
12	0	0	0.2	7
13	−1	−1	0.1	6

the capsule was smeared in advance with soybean oil. The pin bar was dipped in a plate containing the gelatin solution, and then the wet capsules were removed and dried at around 25–27 °C for approximately 30 min. The dried capsules were then removed from the pin bars. The obtained capsules were analyzed for the acidity degree, moisture content, ash content, capsules cap length and body, dimensions, and disintegration time and and compared with commercial capsules.

2.5. Chemical composition

Chemical composition was determined on pangasius fish skin moisture, ash, fat, and protein contents (AOAC, 2005). The crude protein was measured using a Kjeldahl method, while fat content was determined by a Soxhlet method. However, for the gelatin and capsules, only moisture and ash contents were determined.

2.5.1. Yield

The yield was obtained by calculating the ratio of dry gelatin weight to the weight of skin (raw material) before the gelatin extraction (AOAC, 1995).

$$\text{Yield}\,(\%) = \frac{\text{Gelatin Dry Weight}\,(\text{g})}{\text{Raw Material Weight}\,(\text{g})} \times 100$$

2.5.2. Acidity degree

The pH value of gelatin was determined according to Hue et al. (2017) for 1% gelatin (w/v) dissolved in distilled water at around 55–60 ^{0}C under constant stirring for 30 min, followed by cooling down to 25 ^{0} C. The solution pH was directly measured using a pH meter (Metrohm, Switzerland).

2.5.3. Gel strength

(GMIA, 2019) Dry gelatin was put into a bloom jar, and dissolved in 100 mL of distilled water to prepare 6.67% (w/v) solution and periodically stirred at 65 °C. The solution was cooled for 15 min at a room temperature and stored at 10 °C for 17 h. Gel strength or bloom was determined using a gelometer (GCA, Grace Instrument, USA).

2.5.4. Viscosity

Gelatin solution of 6.67% (w/v) was put into an Erlenmeyer flask and brought to 100 mL with distilled water. The dissolved gelatin was heated to 61–62 °C. The viscosity (GMIA, 2019) of the solution was measured by using a Brookfield TV-10 digital viscometer (Toki Sangyo, Japan) with spindle No. 1 (Model LV) at 100 rpm and was expressed in centipoises (cP).

2.5.5. Setting point

The setting point was measured as reported (Nurilmala et al., 2020a). A glass cylinder set at 35 °C and a water bath set at 15 °C were used. Briefly, the gelatin (7.5 g) was dissolved in 50 mL distilled water, and heated in a water bath until the solution temperature reached 35 °C. The gelatin solution was put into the tube. The filter paper was inserted in gelatin solution. Then, and this solution was placed into a glass cylinder, which had been submerged in water at 15 °C. The solution was stirred with a thermometer until the gelatin began to harden as indicated by the filter paper which has stopped moving.

Table 3 Chemical composition of pangasius skin.

Parameter	This experiment	*P. sutchi*[1]	*P. bocourti*[2]
Moisture (%)	58.67 ± 0.53	50.03 ± 0.27	60.86 ± 0.65
Ash (%)	0.11 ± 0.00	4.14 ± 0.18	0.18 ± 008
Fat (%)	13.44 ± 0.31	6.95 ± 0.17	2.19 ± 0.64
Protein (%)	27.54 ± 0.12	30.91 ± 0.28	35.83 ± 2.61

[1] Mahmoodani et al., 2014, [2]Prommajak and Raviyan, 2013.

2.6. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)

According to the method of He et al. (2020), 2 mg gelatin was dissolved in 1 mL of SDS 5% and heated at 85 °C for 1 h, followed by centrifugation for 5 min at room temperature at 12400 rpm, and 20 μL of the supernatants were taken. These were pretreated with SDS at 85 °C for 10 min. As much as 5 μL of the sample solutions were applied to 15% polyacrylamide slab gel and run at 100 V for 3 h. The gel was then soaked in 25 mL of Coomassie Brilliant Blue staining solution for approximately 2 h.

2.7. Fourier transform infrared (FTIR) spectrometry

FTIR analysis was performed according to Dai et al. (2020) to determine the functional groups and presence of the resulting gelatin. Dried gelatin (2 mg) was mashed with KBr powder in a mortar to homogeneity, then put into a pellet mold, compacted and vacuumed in a pellet molding machine. The pellet was put into the cell which was placed in the cell placement chamber and then fired with IR light from an IR-408 infrared spectrophotometer (PerkinElmer, Boston, MA, USA). The histogram was then analyzed to obtain qualitative and quantitative data.

2.8. Capsule weight and dimension measurement

The weight of the capsules was measured using an analytical balance as described in Nurilmala et al. (2020b), while dimensional measurements were carried out for the length and diameter of the capsules using a caliper, after separating the capsule shell into the capsule body and cap.

2.9. Disintegration time

Disintegration time was measured as described in Nurilmala et al. (2020b). Briefly, the capsule shell put in a glass beaker containing 100 mL of distilled water was set at 37 °C for imitating human body temperature. The time for complete solubilization of the capsule shell was defined as the resistance value of the capsule shell in water.

2.10. Statistical analysis

Independent T-test (Ross and Wilson, 2017) was used to analyze the characteristics of capsules from the skin gelatin and commercially available capsules.

3. Results and discussion

3.1. Chemical composition of pangasius skin

The chemical composition is presented in Table 3. The results showed the pangasius skin contained high protein and very low ash, indicating that it is a good source of gelatin. High protein content is needed to obtain higher yield, while low ash content is required to get a good quality of gelatin. The protein content of pangasius skin in this study was slightly lower than those of the two closely related secies, *P. sutchi* (Mahmoodani et al., 2014) and *P. bocourti* (Prommajak and Raviyan, 2013). In contrast, the lipid content was higher than those of the two species. High lipid content is undesirable during gelatin production because of resulting in lower quality (Kim et al., 2020). Low contents of ash, lipid and other impurities are important for the quality of gelatins.

3.2. Optimization of the gelatin extraction procedures

The results of the characteristics of gelatin based on the experimental design are shown in Table 4. There were 13 gelatin formulas from the Design Expert program version 10 with a central composite design (CCD) that were used in this study. The results of the 13 designs showed different results for each response.

The mathematical models for each response are provided in Table 5. The mathematical models for the yield and moisture response followed linear regression, while the gel strength, ash, pH, viscosity and setting point followed quadratic regression. These models were developed after ignoring insignificant variables and were found to be statistically significant with P values < 0.05 and lack of fit value (> 0.05).

3.3. Yield

Yield is a very important factor for gelatin production industry for economic reason. The model equation indicated that the yield was positively correlated with the citric acid concentration and extraction time. The 3D surface plot showed the effect of extraction time and citric acid concentration on gelatin yield (Fig. 1A). The extraction yield increased sharply when the extraction time was prolonged from 6 h to 8 h and the citric acid concentration increased from 0.1% to 0.3%. It is well understood that acid could breakdown collagen structure into subunit chains like α and β as well as high molecular weight components. When the extraction time was prolonged, there will be more breakdown of collagen and increase in the gelatin

Table 4 Characteristics of gelatin based on the experimental design.

Run	X_1	X_2	Yield (%)	Gel strength (bloom)	Moisture content (%)	Ash content (%)	pH	Viscosity (cP)	Setting point (°C)
1	0.3414	7	20.5	250.31	6.29	0.97	5.67	63	23.5
2	0.2	7	14	279.37	6.50	0.30	6.51	57	21
3	0.2	7	18	264.73	6.40	0.96	6.84	67	19.5
4	0.2	5.58	12	220.96	5.82	0.39	6.19	68	20
5	0.0585	7	14.5	169.84	6.31	0.96	6.95	53	21
6	0.3	8	18	220.93	7.09	0.96	6.67	63	21
7	0.2	8.41	21	168.36	6.90	0.96	6.76	71	21
8	0.3	6	16.5	203.30	5.96	0.93	5.54	69	24.5
9	0.1	8	16.5	154.79	6.18	0.95	6.75	68	20
10	0.2	7	18	213.04	5.60	0.96	6.60	66	20
11	0.2	7	16	283.81	5.81	0.40	5.65	62	21.5
12	0.2	7	18	259.02	5.47	0.30	6.33	63	20.5
13	0.1	6	12.5	228.57	5.54	0.96	6.76	69	20

Table 5 Response surface models for processing conditions of gelatin from pangasius skin.

Response	Equation	*P* value
Yield	$Y = 2.227858X2 + 17.4826X1\text{-}2.8697$	0.0018
Gel strength	$Y = -32.7910X_2^2\text{-}2507.8999X_1^2 + 397.0523X_2\text{-}403.1971X_1\text{-}1051.5923$	0.0151
Moisture	$Y = 0.4123X2 + 1.6340X1 + 2.92977$	0.0274
Ash	$Y = 0.1401X2^2 + 25.8633X1^2\text{-}1.8772X2\text{-}0.3625X + 17.8136$	0.0444
pH	$Y = 2.8500X1X2\text{-}0.4370X_2\text{-}23.8379X1 + 10.3392$	0.0080
Viscosity	$Y = 3.5010X2^2\text{-}225.0676X1^2\text{-}37.50000X1X2\text{-}40.6094X2 + 376.4556X1 + 161.9257$	0.0352
Setting point	$Y = 86.6632X1^28.7500X1X2 + 1.4718X2 + 37.8820X1 + 11.4170$	0.0120

yield. The yield of gelatin produced in this study varied between 12% and 21%. Using modeling calculation, the maximum yield was 20.54% at the citric acid concentration of 0.3% and extraction time of 8 h, though the actual experimental yield was 22.50%. The yield was higher than those of blackspotted croaker (*Protonibea diacanthus*) (19.19%), tuna (*Thunnus thynnus*) (18.1%) (Haddar et al., 2011), Nile tilapia (18.1%) (Songchotikunpan et al., 2008), Atlantic salmon (*Salmo salar*) (11.3%) and Atlantic cod (*Gadus morhua*) (10.1%) (Arnesen and Gildberg, 2007). In addition to the extrinsic factors such as extraction time, pH, and temperature, the variation of gelatin yield is also caused by the intrinsic factors including types of fish, collagen content, fish age, and organ sources.

3.4. Gel strength

Gel strength is a measure of the hardness, stiffness, strength, and compressibility of the gel at a certain temperature and is also influenced by concentration and molecular weight (Irwandi et al., 2009). The strength of the gelatin gel in this study was 154.79–283.81 bloom. The model equation showed that gel strength was positively correlated with the extraction time but negatively correlated with the citric acid concentration, the quadratic effect of extraction time, and the quadratic effect of citric acid concentration. Surface contour images of the gel strength response can be seen in Fig. 1B. The highest gel strength was found in the citric acid concentration of 0.25% with an extraction time for 7 h. The gel strength of pangasius skin gelatin was higher than those of perch (Monsur et al., 2014) and unicorn leatherjacket (*Aluterus monoceros*) counterparts (Hanjabam et al., 2015). Gel strength is the most important attribute of gelatin and determines the quality of produced gelatin. The application of gelatin was determined by the range of gel strength values. Usually, the gel strength of commercial gelatins ranges from 100 to 300 bloom, but gelatins with gel strength of 250–260 bloom are the most desirable and suitable for a wide range of applications in the food industry especially in the processing of jellies, canned meat, marshmallows and yoghurts. Some species of warm-water fish gelatins have been reported to exhibit high gel strengths, close to that of porcine gelatin, while the gelatin from cold water fish species have poorer physical properties. This is due to a lower content of the amino acids, proline and hydroxyproline in the gelatin from cold water fish species. Factors that affect gel strength are differences in the contents of proline and hydroxyproline as well as the distribution of molecular weight and the type of pretreatments such as the concentration of acid used (Hue et al., 2017).

3.5. Moisture content

Moisture content is an important parameter for determining acceptability, appearance, texture, quality of materials, and durability of materials. The moisture content of gelatin obtained in this study was in range of 5.47–7.08%. The model equation showed that the moisture content is positively correlated with the concentration of citric acid and the extraction time. Surface contour of the gelatin moisture content can be seen in Fig. 1C. The highest moisture content was found in

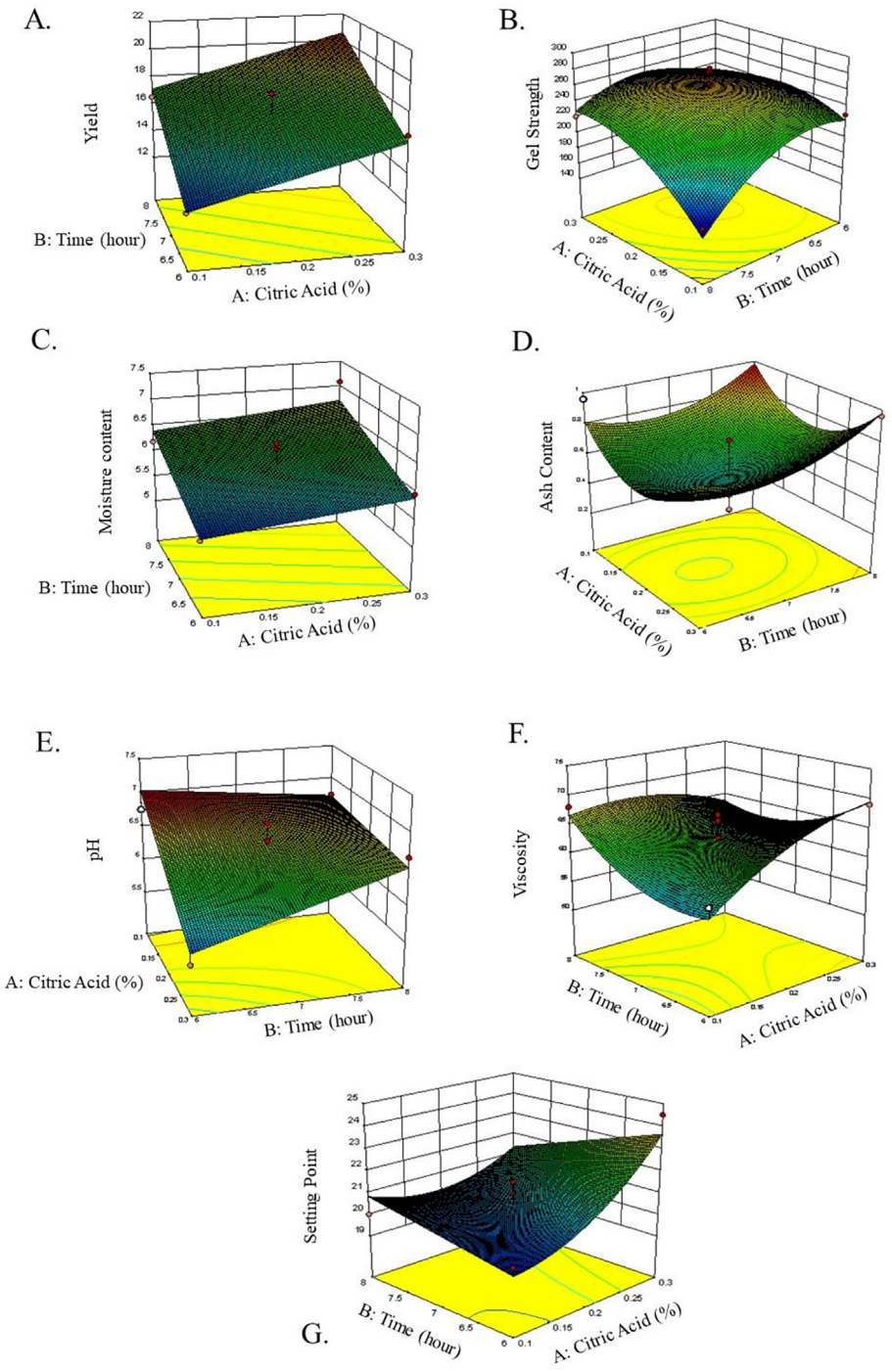

Fig. 1 Response surface plots of the effects of citric acid concentration and extraction time on yield (A), gel strength (B), moisture (C), ash (D), pH (E), viscosity (F), and setting point (G).

the citric acid concentration of 0.3% with an extraction time of 8 h. The lowest moisture content was in the blue area or the area with 0.1% citric acid with an extraction time of 6 h. The difference in moisture content may be influenced by the drying process using an oven and drying time.

3.6. Ash content

The model equation showed that the response of ash content was positively correlated with the quadratic effect of the citric acid concentration, the quadratic effect of the extraction time but was negatively correlated with the linear effect of citric acid concentration, reducing the linear effect of extraction time. The surface contours and 3D surfaces in Fig. 1D showed that the highest ash content was in the red color or in the citric acid area with a concentration of 0.1% with an extraction time of 6 and 8 h and a citric acid concentration of 0.3% with the extraction time of 6 and 8 h. The lowest ash content was shown in the blue area or the area with 0.2% citric acid with extraction time of 6.5–7 h.

3.7. pH

Measurement of the pH value of the gelatin solution is important, because the pH of the gelatin solution affects other gelatin properties such as viscosity, gel strength, and also affects the application of gelatin in the product. The pH of the gelatin produced in this study was 5.54–6.95%. Different values of acid strength effect on the number of H^+ ions that is bound to proteins, then contributing the acidity level of the gelatin produced. The model equation showed that the pH response is positively correlated with the interaction of citric acid concentration and extraction time, but is negatively correlated with the linear effect of citric acid concentration as well as extraction time. Surface contours of pH can be seen in Fig. 1E, which shows that the highest pH is found in the red color or in the citric acid region with a concentration of 0.1% with an extraction time of 8 h. The lowest pH was in the blue area or the area with 0.3% citric acid with an extraction time of 6 h.

3.8. Viscosity

Viscosity is the second most important physical parameter for gelatin. The gelatin viscosity produced in this study was 53–71 mPa-s (cP). The model equation showed that the viscosity response value was positively correlated with the linear effect of citric acid concentration and the quadratic effect of the extraction time concentration, but was negatively correlated with the linear effect of extraction time, the interaction effect of citric acid concentration and extraction time, and the quadratic effect of citric acid concentration. Surface contours of viscosity can be seen in Fig. 1F, which showed that the highest viscosity was found in the red color or in the citric acid region with a concentration of 0.3% with an extraction time of 6 h. The lowest viscosity was shown in the blue area or the area with 0.1% citric acid with an extraction time of 6 h.

3.9. Setting point

The setting point is the temperature required when the gelatin solution in a certain concentration forms a gel. The setting point of gelatin in this study was 19.5–24.5 °C. The equation of the model showed that the setting point is positively correlated with the quadratic effect of the extraction citric acid concentration and the linear effect of extraction time, but was negatively correlated with the linear effect of the citric acid concentration and the interaction of citric acid concentration and extraction time. The surface contours of viscosity is presented in Fig. 1G. The highest setting point was found in the red color or in the citric acid area with a concentration of 0.3% with an extraction time of 6 h. The lowest setting point was shown in the blue area or the area with 0.1% citric acid with an extraction time of 6 h. The difference in setting point can be caused by differences in the contents of glycine and hydroxyproline in the gelatin, which resulting in the loss of hydrogen bonds in the gelatin solution. In this study, glycine conten was 30.17%, while hydroxyproline was 1.33% (data not shown). The setting point is also influenced by the concentration of gelatin in solution, pH, and molecular weight (Mad-Ali et al., 2017).

3.10. Optimal state validation

Optimal conditions were found at 0.3 % citric acid concentration and 8 h of extraction time based on selection from all response variables of gelatin. Validation was carried out by comparing the optimal prediction results given by the software with the actual value. The optimal prediction value given by the software will be followed by a 95% confidence interval (CI). The comparison of the actual response value with the predicted response value is shown in Table 6.

The actual value of the yield response was greater than the predicted value, but was still in the 95% CI scale. The actual value of the response gel strength, ash content, pH, viscosity, and setting point were smaller than the respective predicted values. The results obtained were still within the 95% CI scale. The actual value of moisture content was smaller than the predicted value and was not included in the 95% CI scale. The value of moisture obtained still met the standard of GMIA 2019.

3.11. SDS-PAGE and FTIR analysis

SDS-PAGE and FTIR analysis were carried out to determine the optimum condition. The gelatin preparation in this study showed the bands of the subunits, namely, α1, α2, β and γ chains based on the SDS-PAGE patterns (Fig. 2). The molecular weights of these subunits were 82 kDa for α1, 97 kDa for α2, 211 kDa for β, and 276 kDa for γ, respectively. The α1, α2 and β chains were the typical triple helix chains consisting of

Table 6 Comparison of the actual response value with the predicted response value.

Response	Actual values	Predicted values	95% CI	
			Low	High
Yield (%)	22.50 ± 0.00	20.54	18.52	22.57
Gel strength (bloom)	191.85 ± 8.19	228.21	185.28	271.15
Moisture content (%)	5.19 ± 0.46	6.71	6.22	7.20
Ash content (%)	0.79 ± 0.05	0.96	0.69	1.23
pH	6.07 ± 0.16	6.53	6.07	6.99
Viscosity (mps)	62 ± 3.21	63.97	58.02	69.93
Setting point (°C)	20.67 ± 0.33	21.29	19.80	22.78

Note: CI = Confidence Interval.

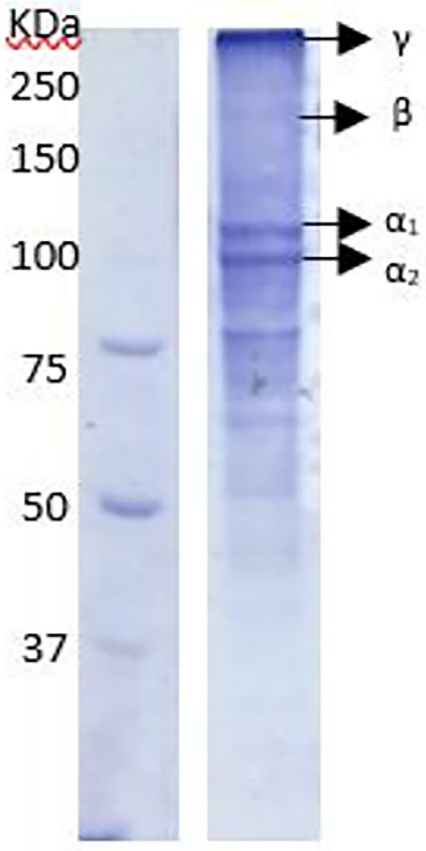

Fig. 2 SDS-PAGE pattern of the gelatin preparation.

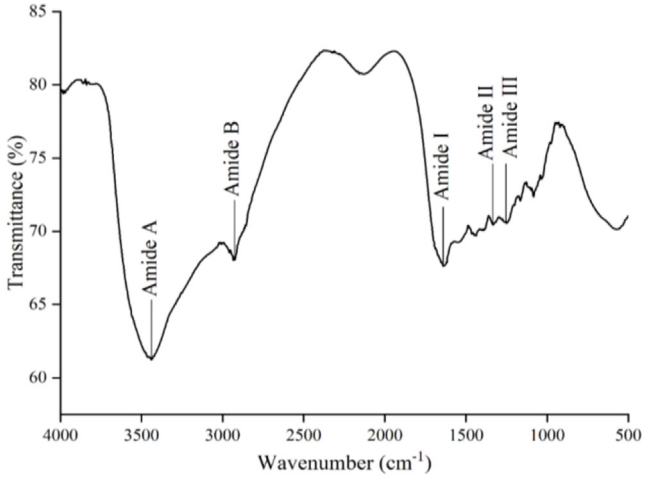

Fig. 3 Fourier transform infrared (FTIR) of fish skin gelatin.

collagen molecule, while the γ chain forms the double structure of the triple helix. The range of molecular weights corresponds to the molecular weight of gelatin in general. Boran et al. (2010) reported that the range of molecular weights was 80–125 kDa for the α chain, and 160–250 kDa for the β chain.

FTIR analysis was carried out to compare the gelatin preparation of this study with that reported by Maryam et al. (2019). Carbonyl (C = O), amine (NH), and hydroxyl (OH) groups are generally present in the gelatin structure and give rise to five main peaks, namely, amide A (the wavenumber region from 3440 to 3201 cm^{-1}), amide B from 3000 to 2923 cm^{-1}), amide I (from 1698 to 1633 cm^{-1}), amide II (from 1543 to 1447 cm^{-1}) and amide III (the wave number region at 1365 to 1200 cm^{-1}) (Shahvalizadeh et al. 2021; Ashrafi et al. 2023). As shown in Fig. 3, the results of this study showed that there were five peaks, namely, the wavenumbers at 3440; 2933; 1639; 1437 to 1332; 1250 cm^{-1} were the regions of amide A, amide B, amide I, amide II and amide III, respectively.

Table 7 Characteristics of hard capsules.

Specification	This study	Commercially available capsules
Body length (mm)	17.88 ± 0.08[a]	17.90 ± 0.00[a]
Body diameter (mm)	6.54 ± 0.01[a]	6.80 ± 0.14[a]
Cap length (mm)	10.63 ± 0.22[a]	10.62 ± 0.12[a]
Diameter cap (mm)	7.05 ± 0.06[a]	7.27 ± 0.03[a]
Capsule weight (g)	86.80 ± 2.00[a]	99.00 ± 1.41[b]
Moisture (%)	14.58 ± 0.55[a]	15.35 ± 0.07[a]
Ash content (%)	0.58 ± 0.28[a]	1.15 ± 0.20[a]
Acidity degree (pH)	6.18 ± 0.02[a]	5.75 ± 0.10[b]
Disintegration time (min)	1.46 ± 0.00[a]	5.90 ± 0.28[a]

Note: The same superscript letters do not show any significant difference ($p > 0.05$).

3.12. Characteristics of hard capsules

The capsules made in this study were those with size 0. The characteristics of the hard capsules are shown in Table 7.

The gelatin capsules made from pangasius skin gave the characteristics that were in accordance with the commercial capsules based on the dimensions, weight, moisture content, ash content, pH, and disintegration time. The capsules made from fish gelatin were found to have similar properties as the commercial capsules. Fig. 4 shows the process of production

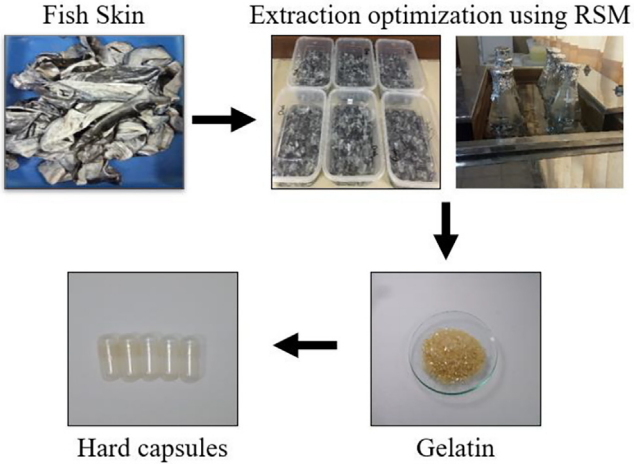

Fig. 4 Preparation procedures of skin gelatin and hard capsules.

pangasius fish skin gelatin using RSM and the application for hard capsules.

4. Conclusion

Pangasius fish skin can be made into high quality gelatin with characteristics in accordance with GMIA standard (2019) based on the gel strength, viscosity, acidity (pH), moisture content and ash content. Optimization of the process for producing gelatin from pangasius skin using the RSM method resulted in the optimum response at 0.3% citric acid and extraction time for 8 h for the response of yield, gel strength, moisture, ash content, pH, viscosity and setting point. In addition, the obtained gelatin was found to be the good source for producing hard capsules for Moslem community.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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