Process Optimization and Properties of Crude Gelatin Extracted from Tannery Bovine Hide

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Abstract

Underutilized bovine hide from the tannery industry was investigated for the optimum conditions for extraction and the properties of gelatin. Central composite design and response surface methodology were employed to determine the effect of temperature of extraction (60°C to 80°C) and time of extraction (6 h to 48 h) on the yield and the gel strength of crude gelatin. The optimum condition was found to be extraction from delimed skin with 0.15 M acetic acid at 70°C for 6 h, which resulted in a gelatin yield of 4.78% and gel strength of 265.45 g. The crude gelatin consisted of low molecular weight components with protein bands between 6 kDa and 38 kDa. The overall properties were found to fall within the product standard, though the values of gel strength, viscosity, and foaming properties were lower than the values for commercial halal bovine gelatin. It can be concluded that tannery bovine hide could be a potential raw material for production of low bloom gelatin.

Keywords: bovine hide, gelatin, tannery industry

1. Introduction

The tannery industry in Thailand has a total annual production capacity of 360,000 tons of leather per year, and about 90% of the hides are obtained from cows and buffaloes. The underutilized hide obtained after the deliming process is the primary by-product of tanneries (Thai Tanning Industry Association, 2016). The hide could be a new alternative raw material for producing gelatin. Consequently, the waste treatment and disposal cost in the tannery industry could be reduced. Generally, gelatin is made from untreated materials such as hides and bones (Jayathilakan *et al*., 2011). The optimal condition for preparation of gelatin from tannery by-products needs to be investigated as the quality of underutilized hide could be affected by the liming process at tanning factories (Babin and Dickinson, 2001).

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In previous studies, it has been reported that extraction of gelatin from bovine tendon by water provided yields of 55–60% and gel strength of 350–410 bloom (Mokrejs *et al*., 2009). The collagen from bovine limed split wastes, which was pretreated with hydrochloric acid solution and extracted using acetic acid solution with 2% pepsin, had a yield of 41.31% collagen protein (Li *et al*., 2008). Extraction of gelatin from tannery bovine hides by water at 64–84°C with a total extraction time of 12 h brought forth a gelatin yield of 13.6% (Mahmood *et al*., 2008). However, information related to the properties was not available. To our knowledge, extraction of this raw material with weak acids, such as acetic acid, has not been presented yet.

Compared to water, using a weak acid in bovine hide gelatin extraction may reduce the treatment time. Therefore, this study aimed to investigate the optimal condition for acid extraction of gelatin from tannery bovine hides by using response surface methodology (RSM) to utilize the hide waste and reduce its disposal cost in tannery factories. The functional properties, including gel strength, color, turbidity, viscosity, foaming properties, and molecular weight distribution of the crude gelatin, were compared with those of commercially available bovine gelatin. The sorption isotherm of dry gelatin was also investigated.

2. Materials and Methods

2.1 Materials

Underutilized bovine hide was provided by the Chai Watana Tannery Group (Samutprakarn, Thailand). The hide sample was pre-salted, treated with calcium hydroxide, and then delimed at the tanning factory. The delimed skin sample was frozen and transported to the Division of Food Science and Technology, Chiang Mai University, Chiang Mai, within 24 h.

Commercial halal bovine gelatin was obtained from the Union Science (Thailand). The acrylamide and sodium dodecyl sulfate (SDS) were purchased from LOBA Chemie (India). The bromophenol blue and Coomassie Brilliant Blue were from LabChem Inc. (USA). The N,N,N',N'-tetramethylethylenediamine (TEMED) and bis-acrylamide were obtained from Merck Millipore (Germany). The pre-stained protein standard was purchased from Novex (Germany). All the chemicals and reagents used were of analytical grade.

2.2 Pretreatment of bovine hide

The bovine hide samples were defrosted, thoroughly washed with tap water before cutting into 2.5 × 2.5 cm^2 pieces (Figure 1), and stored at -18°C in polyethylene bags until use.

Prior to extraction, the hides were pretreated according to the method of Lassoued *et al.* (2014) and Liu *et al.* (2015), with slight modification to remove non-collagenous proteins. In brief, the hides were soaked in 1.5% NaOH solution with a skin-to-alkaline solution ratio of 1:10 (w/v) at room temperature for 40 h. The alkaline solution was changed every 10 h during soaking. Then, the alkaline-pretreated skin was thoroughly washed with water until the pH of the washing water turned to neutral.

Figure 1 The bovine hide samples.

2.3 Optimization of gelatin extraction

2.3.1 Experimental design

Response surface methodology (RSM) was used to optimize the condition for gelatin extraction. The experiment was based on a central composite design with four axial points and an α value of 1.2. The extraction temperature (°C, X_1) and the extraction time (h, X_2) were independent variables. The levels of the independent variables are shown in Table 1. On the other hand, the gelatin yield (%, Y_1) and the gel strength (g, Y_2) were dependent variables. The experimental design consisted of 13 treatments, as shown in Table 2.

Table 1 Levels of Independent Variables in Central Composite Design for Gelatin Extraction

Independent variable	Symbol	Level				
		$-\alpha$	−1	0	+1	$+\alpha$
Temperature (°C)	X_1	58	60	70	80	82
Time (h)	X_2	1.8	6	27	48	52

Table 2 Treatments of Central Composite Design and Response Values of Gelatin Extraction

Run no.	Coded level of variable		Response value*	
	Temperature (°C, X_1)	Time (h, X_2)	Yield (%, Y_1)	Gel strength (g, Y_2)
1	0	0	8.23 ± 0.04	215.96 ± 17.08
2	0	0	8.33 ± 0.09	229.13 ± 18.71
3	-1.2	0	7.24 ± 0.03	232.87 ± 23.03
4	0	+1.2	12.12 ± 0.01	90.84 ± 31.32
5	-1	+1	8.50 ± 0.04	217.78 ± 10.58
6	0	0	8.47 ± 0.03	237.24 ± 16.62
7	+1	+1	11.79 ± 0.01	8.33 ± 2.29
8	0	0	8.14 ± 0.08	221.61 ± 23.63
9	-1	-1	3.49 ± 0.02	250.75 ± 17.85
10	+1.2	0	9.72 ± 0.02	84.02 ± 18.25
11	0	-1.2	2.09 ± 0.03	267.96 ± 18.25
12	0	0	8.53 ± 0.04	208.06 ± 7.39
13	+1	-1	8.60 ± 0.00	137.38 ± 9.44

Note: * Expressed as mean ± standard deviation of triplicate determination.

2.3.2 Extraction of crude gelatin

Underutilized bovine hide gelatin (UBHG) was obtained by mixing the pretreated skin, discussed in section 2.2, with 0.15 M acetic acid at a ratio of 1:4 (w/v). The samples were incubated in a water bath (SV2945 model, Memmert, Germany) with continuous shaking at 100 rpm using the extraction temperatures and times shown in Table 2. After filtration through a piece of double-layered cheese cloth, the samples were clarified by mixing with 5% (w/v) activated carbon and kept at 60°C for 1 h before filtering through a Whatman No. 4 filter paper and then centrifuged at 2,500× g (Z206A model, Hermle Labortechnik, Germany) for 15 min at 25°C. The supernatant was collected and dried at 60°C using a convection oven (ULM500 model, Memmert, Germany). The dried gelatin was kept at 4°C in a sealed container for further experiments (Figure 2).

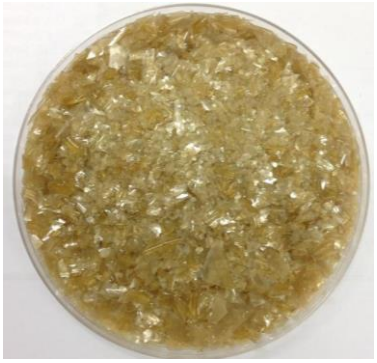

Figure 2 The crude gelatin flakes.

2.3.3 Determination of crude gelatin yield

The yield of crude gelatin was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{weight of dried gelatin (g)} \times 100}{\text{wet weight of fresh skin before pretreatment (g)}}$$

2.3.4 Determination of crude gelatin gel strength

The gel strength was analyzed according to the method of Zhou and Regenstein (2004), with a slight modification. The gelatin solution (6.67% w/v) was prepared by dissolving dried gelatin in distilled water, and the solution was heated at 60°C for 1 h. Then, the gelatin solution was filled in a 30 mm diameter × 15 mm height cup and kept at 4°C for 16 h to 18 h. The gel strength, that is, the maximum force required for penetration, was measured by a texture analyzer (TA.XT Plus model, Stable Micro Systems, England) using a 12.7 mm diameter plunger (P/0.5R probe) at 0.5 mm/s compression rate and 4 mm penetration depth at room temperature.

2.3.5 Regression analysis and optimization

The Design-Expert statistical software (version 6.0.2, Stat-Ease, Inc., USA) was used for regression analysis and optimization of the extraction condition of gelatin. The following quadratic polynomial equation was used as the regression model:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_1^2 + \beta_4 X_2^2 + \beta_5 X_1 X_2,$$

where Y is the measured response variable; β_0 is constant; β_1, β_2, β_3, β_4, and β_5 are regression coefficients; and X_1 and X_2 are the extraction temperature and time, respectively. The analysis of variance of the regression model and the correlation coefficient (R^2) were investigated. The optimum conditions were calculated based on the gelatin yield and the gel

strength. Verification of the model was performed by extraction of gelatin at optimum conditions in triplicate.

2.4 Determination of physical and chemical properties of dried gelatin

Crude gelatin was extracted at the optimum condition and dried at 60°C by using a convection oven (ULM500 model, Memmert, Germany) until the recommended moisture content of less than 13% was obtained (D'Cruz and Bell, 2005). The following properties of the dried gelatin were compared with those of commercial bovine gelatin.

2.4.1 Proximate analysis

The moisture, ash, lipid, and protein contents of the gelatin samples were examined by the AOAC methods (AOAC, 2000). All the measurements were performed in triplicate.

2.4.2 Viscosity

The viscosity was analyzed according to the method of Prommajak and Raviyan (2010), with a slight modification. Gelatin solution (6.67% w/v) was prepared and allowed to equilibrate at 60°C for 30 min before measuring the viscosity with a Brookfield viscometer (SVDV-II+ Pro EXTRA model, Brookfield Engineering Laboratories, USA) using an S18 probe at 200 rpm. The measurements were performed in triplicate.

2.4.3 Color

For color measurement, the samples were prepared by following the sample preparation method described in the determination of the gel strength (section 2.3.4). The color was measured in the L*, a*, and b* color space system using a Chroma Meter (CR-410 model, Konica Minolta Sensing, Japan).

2.4.4 Turbidity

The turbidity of the 6.67% (w/v) gelatin solution was determined by measuring the transmittance of the 10 mm path length at 620 nm using a spectrophotometer (GENESYS 10S UV Scanning model, Thermo Scientific, Germany) (Schrieber and Gareis, 2007).

2.4.5 Foaming properties

The foaming properties were measured according to the method of Aewsiri *et al.* (2008), with a minor modification. One hundred milliliters of 2% (w/v) gelatin solution was homogenized at 10,000 rpm for 5 min using a homogenizer (T-50 digital Ultra-Turrax model, IKA, Germany) at room temperature and then rapidly transferred into a 250 ml cylinder. The foam volume was recorded instantly after homogenization and after leaving at room temperature for 30 min. Foam expansion (FE) and foam stability (FS) were calculated using the following equations:

$$\text{Foam expansion (FE)} = \frac{\text{Total volume after homogenization X 100}}{\text{Total volume before homogenization}}$$

$$\text{Foam stability (FS)} = \frac{\text{Foam volume after 30 min X 100}}{\text{Foam volume at 0 min}}$$

2.4.6 Protein pattern

By following the method described by Laemmli (1970), with a slight modification, the protein pattern was analyzed by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). The gelatin powder (1 g) was dissolved in a buffer solution of 0.6% (w/v) Tris-HCl, 10% (v/v) glycerol, 1% (w/v) SDS, and 0.4% (w/v) bromophenol blue, heated at 95°C for 4 min, cooled to room temperature, and centrifuged at 13,000 rpm for 10 min to remove the undissolved debris. Ten to fifteen microliters of the supernatant (of about 5 µg/µL of protein content) was analyzed for the protein pattern by electrophoresis (PowerPac HC model, Bio-Rad Laboratories, USA) by using polyacrylamide gels containing a 10% separating gel and a 4% stacking gel. Firstly, the gel was stained by a staining solution of 0.15% (v/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) glacial acetic acid. Then, the gel was destained by a reagent containing 10% (v/v) methanol and 7.5% (v/v) glacial acetic acid for 2 or 3 times until clear bands were obtained. The pre-stained protein standard (with the molecular weight ranging from 3 kDa to 198 kDa) was used as the molecular weight marker.

2.4.7 Sorption isotherm

The sorption isotherm of the UBHG was measured by the gravimetric method (Boonyai, 2007). The UBHG sample (2 to 3 g) was placed in vacuum desiccators at room temperature (25±5°C). The atmosphere within the vacuum desiccators was modified to have relative humidity (RH) between 0.11 and 0.52 by using saturated salt solutions, including LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, and $Mg(NO_3)_2$ (Rizvi, 1986; Roos, 1995). The samples were weighed every 24 h until two values of consecutive weighing were equal, supposing that an equilibrium condition was achieved. The equilibrium moisture content was analyzed by the AOAC (2000) method and the water activity was measured by a water activity meter (Series 3 model, AquaLab, USA). The sorption isotherm was a plot between moisture content and water activity.

2.5 Statistical analysis

The data analysis was performed by SPSS Statistics (version 17.0, IBM, USA). All the experiments were performed in triplicate. One-way analysis of variance (ANOVA) was used to evaluate the difference between the means.

3. Results and Discussion

3.1 Optimum conditions for extraction of gelatin

The response surface methodology (RSM) approach was employed to study the effect of the extraction temperature (60°C to 80°C) and the extraction time (6 h to 48 h) on the yield and the gel strength of the UBHG. The relationships between the factor variables and the response variables were observed to have significantly fitted with the quadratic regression models, as shown in Table 3.

Table 3 Regression Models of Gelatin Extraction from Underutilized Bovine Hide Wastes

Regression model*	R^2	P-value
Yield (%) = $-7.26 + 0.17X_1 + 0.14X_2$	0.86	<0.0001
Gel strength (g) = $-1533.21 + 55.03X_1 + 8.81X_2 - 0.42X_1^2 - 0.06X_2^2 - 0.11X_1X_2$	0.96	<0.0001

Note: * X_1 and X_2 are the extraction temperature and the extraction time, respectively.

The regression models of gelatin yield and gel strength were highly significant (p<0.01). The adjusted correlation coefficients (R^2) of the two models were 86% and 96%, respectively (Table 3). Both the models could be considered reliable and used to determine the optimal condition for gelatin extraction.

The extraction time and the extraction temperature had an influence on the gelatin yield and the gel strength. In general, the gelatin yield linearly increased as the extraction temperature and time increased (Figure 3a). When gelatin was extracted at higher temperatures, the hydrogen bond between the α-chains in the native mother collagen was disrupted. The triple helix configuration turned amorphous, and the gelatin could be extracted into the solution effectively, resulting in higher gelatin yield (Sinthusamran *et al.*, 2014).

In contrast, the gel strength decreased as the extraction temperature and the extraction time increased (Figure 3b). However, the relation between the factor variables and the response variables was not linear. The gel strength was observed to slowly decrease in treatments at low heat, and the rate of the gel strength reduction was observed to increase at higher extraction temperatures and times. As the chain length of molecules is one of the major

factors that determines the gelatin-processing condition (Kaewruang *et al*., 2013), the reduction in the gel strength could be due to degradation of gelatin in treatments at higher heat.

In this study, the maximum yield was 13.06%, lower than those of 55–60% obtained from bovine tendon waste (Mokrejs *et al*., 2009) and 13.6% obtained from tannery bovine hide waste (Mahmood *et al*., 2008). Conversely, the yield was higher than the yield of 4.17% obtained from delimed bovine split waste (Li *et al*., 2008). Nevertheless, the gelatin yield of 13.06% was the highest value obtained under the conditions employed in this study. As evident from Figure 3a, it could be extrapolated that gelatin yield could be increased at higher extraction temperatures or with prolonged extraction times.

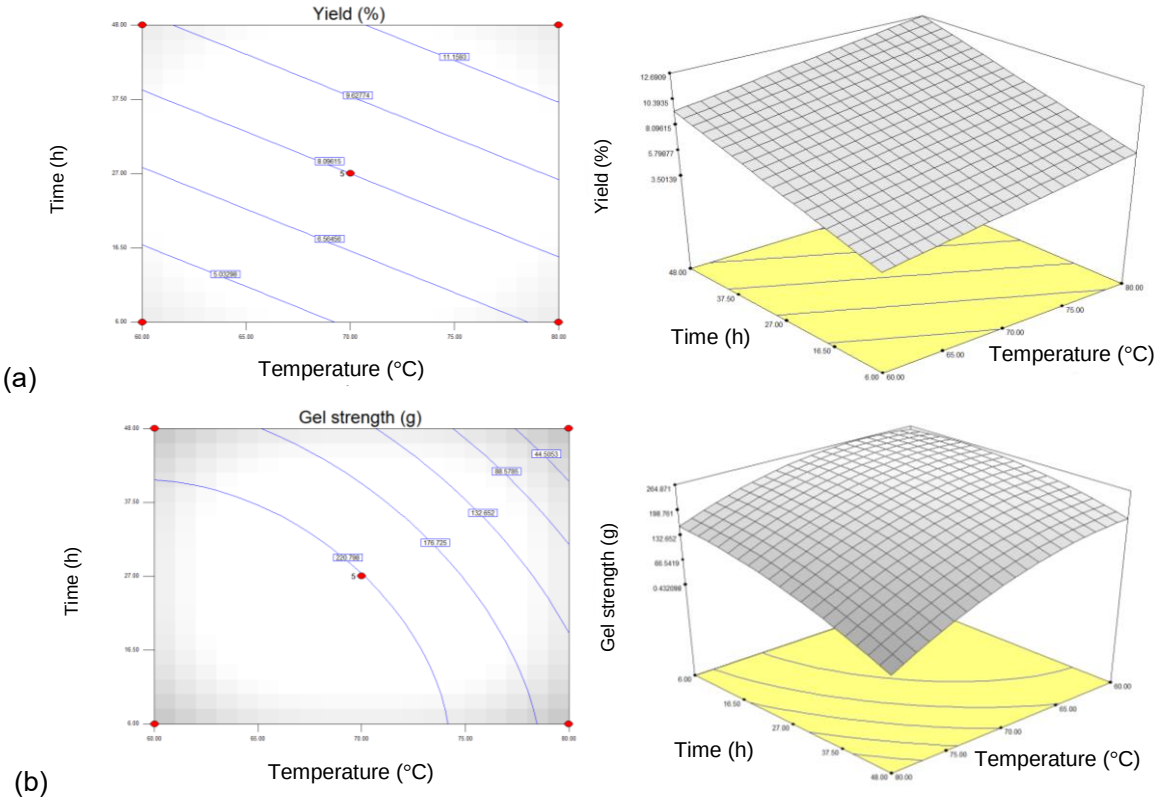

Figure 3 Contour plots and surface plots of (a) gelatin yield and (b) gel strength as functions of temperature and time of gelatin extraction.

Depending on the purpose, the optimum conditions for extraction vary. If the extraction is focused on achieving the maximum gelatin yield, the optimum conditions for extraction would be 80°C for 48 h. This condition brought forth 13.06% gelatin yield. On the other hand, the optimum condition for attaining the maximum gel strength would be 63°C for 12 h, which provided a gel strength value of 280.62 g. The suggested condition is that industrial productivity could be optimized by a balance between gelatin yield and gel strength at the minimum extraction time, which is extraction at 70°C for 6 h. This condition delivered the

predicted gelatin yield of 5.48% and gel strength of 265.39 g (Table 4). The low gelatin yield may be due to the fact that high amounts of collagen molecules were not extracted into gelatin during the optimal extraction, although the highest yield obtained in this study was 13.06%. This indicates that the skin pretreatment should be further studied to make collagen molecules ready to be extracted, as should be extraction of gelatin by peptidase enzyme, which would provide higher gelatin yield.

Accordingly, the optimum condition of 70°C for 6 h was chosen for the production of the gelatin sample for comparing its functional properties with those of commercial halal bovine gelatin (HBG). The actual and the predicted values of the gelatin yield and the gel strength obtained by this condition were not statistically significantly different, which indicates the usability of the models.

Table 4 Actual and Predicted Values of Yield and Gel Strength of Gelatin Extracted at Optimum Conditions

Criteria	Optimum condition		Response value*			
	Temp. (°C)	Time (h)	Yield (%)		Gel strength (g)	
			Actual	Predicted	Actual	Predicted
Maximum yield	80	48	11.79±0.01	13.06	8.34±2.29	43.43
Maximum gel strength	63	12	4.64±0.01	5.13	237.84±34.50	280.62
Maximum yield, Maximum gel strength, Minimum extraction time	70	6	4.78±0.03	5.48	265.45±18.34	265.39

Note: Expressed as mean ± standard deviation of triplicate determination.

3.2 Proximate analysis

The compositions of the crude underutilized bovine hide gelatin (UBHG) and the purified HBG were noticeably different ($p \leq 0.05$). The crude UBHG had darker color (Figure 2) with lower protein content, but higher lipid and ash contents (Table 5) because HBG was purified using a separator and an ion exchanger to reduce the lipid and the ash contents (Schrieber and Gareis, 2007), while the UBHG was purified only by activated carbon. Thus, further purification is recommended to lighten the color and to reduce excess amounts of lipid and ash. The recommended ash content of commercial gelatin for Thai industrial standard is less than 2% (The Thai Industry Standard Institute, 2005). Several clarification processes have been reported for the purification of crude gelatin (Ahmad and Benjakul, 2011).

Table 5 Proximate Compositions of UBHG and HBG

Composition (% wet basis)	UBHG	HBG
Moisture	$8.20^{ns} \pm 0.08$	$7.61^{ns} \pm 0.39$
Protein	$61.57^{b} \pm 0.51$	$79.02^{a} \pm 1.42$
Lipid	$10.21^{a} \pm 0.07$	$0.46^{b} \pm 0.30$
Ash	$11.45^{a} \pm 0.24$	$0.48^{b} \pm 0.05$

Note: The results are expressed as mean ± standard deviation of triplicate determination. ns indicates insignificant difference between the UBHG and the HBG values. The nitrogen-to-protein conversion factor was 5.55. Having different superscript letters in the same row indicates statistically significant difference between means ($p \leq 0.05$).

3.2.1 Gel strength

The gel strength of gelatin depends on its intrinsic characteristics, namely amino acid composition, ratio of α-chain, amount of β-component, and molecular weight distribution (Lassoued *et al*., 2014; Sarbon *et al.,* 2013). The three-dimensional network of the gelatin structure containing shorter polymer chains has lower gel strength (Whisyler and Daniel, 1990). Thus, the lower gel strength ($p \leq 0.05$) of the UBHG (Table 6) suggests that the liming process of the hides had noticeably broken the polymer chains and the protein structure within the UBHG sample. In addition, the lower gel strength of the UBHG was partly due to the lower protein content (Table 5). Commercially, gelatin is classified by its gel strength into high (220–300 g), medium (150–220 g), and low (<150 g) bloom (Johnston-Banks, 1990). Therefore, UBHG could be classified as a low bloom gelatin. This gelatin type could be used in encapsulation technology.

Table 6 Physical Properties of UBHG and HBG

Property	UBHG	HBG
Gel strength (g)	$144.61^{b} \pm 0.17$	$542.32^{a} \pm 0.55$
Color		
L*	$60.96^{b} \pm 0.06$	$69.32^{a} \pm 0.34$
a*	$2.31^{a} \pm 0.03$	$1.91^{b} \pm 0.04$
b*	$22.87^{b} \pm 0.18$	$26.47^{a} \pm 0.35$
Turbidity ($\%T_{620\ nm}$)	$78.75^{b} \pm 0.37$	$97.40^{a} \pm 0.14$
Viscosity (cP)	$3.32^{b} \pm 0.01$	$9.93^{a} \pm 0.16$
Foam expansion (%)	$120.00^{b} \pm 0.00$	$150.00^{a} \pm 0.00$
Foam stability (%)	$22.50^{b} \pm 1.18$	$75.33^{a} \pm 2.83$

Note: The results are expressed as mean ± standard deviation of triplicate determination. Having different superscript letters in the same row indicates statistically significant difference between means ($p \leq 0.05$).

3.2.2 Viscosity

The viscosity of the UBHG was lower than that of the HBG (P≤0.05) (Table 6). The low viscosity of UBHG could be due to the occurrence of low molecular weight peptide chains (Figure 4) through hydrolysis of the protein chain during the liming process of the hides (Norziah *et al*., 2014; Rafieian *et al*., 2015). The standard viscosity of edible gelatin is 2 cP for type B gelatin (pH 5), 4.5–6 cP for the hard capsule, 2–3.5 cP for the pharmaceutical tablet, and 7.8–9.5 cP for the photographic gelatin (GMIA, 2012). The viscosity value of UBHG (3.32 cP) is acceptable for food and pharmaceutical production.

3.2.3 Color and turbidity

The L* and the b* values of the UBHG were lower than those of the HBG, while the a* value of the UBHG was higher than that of the HBG (Table 6). The results showed that the UBHG had a brownish-to-yellow color, whereas the color of the HBG was pale yellow. Generally, the color of gelatin depends on the raw material and the processing condition (Jakhar *et al*., 2012). The difference in the color could be because SO_2 was used to stabilize the microbiological status and lighten the color of gelatin during commercial production (Schrieber and Gareis, 2007). However, the functional properties of gelatin are not affected by its color (Ockerman and Hansen, 1988).

The turbidity study indicates the presence of significant amounts of residual of dirt and small debris after the deliming at the tanning factory. The insoluble impurities that formed the emulsion, dispersion, or isoelectric haze could have affected the turbidity of gelatin.

3.2.4 Foaming properties

Foam formation ability is an essential property for production of certain foods such as marshmallows (Lassoued *et al*., 2014). The foaming expansion (FE) and the foaming stability (FS) of the HBG were higher than those of the UBHG (p≤0.05) (Table 6). The UBHG was produced from the underutilized bovine hide that was pretreated by the chemical solution, while the HBG was from commercial bovine hides. This study confirmed that the foaming properties of gelatin, depending on the composition and the structure of gelatin at the air and the water interface, were affected by the source of the raw material (Zayas, 1997). The foaming properties of the UBHG were noticeably lower as the solution contained a lower concentration of protein (Table 5) and had lower viscosity (Table 6). It has been reported that foaming properties depend on the binding of molecules at the junction zone, in which polar units bind with the hydrophilic solution and nonpolar ones bind with the hydrophobic solution (Sanchez-Vioque *et al*., 2001). Formation of multilayer cohesive protein films at the interface could be encouraged by increasing the protein concentration and the viscosity of the solution (Lassoued *et al*., 2014; Halling, 1981). The processing conditions also evidently affect the

foaming properties. Gelatin extracted in treatments at lower heat would have bigger molecules, which can hold more air bubbles within the matrix than gelatin with smaller molecules (Karim and Bhat, 2009).

3.2.5 Protein pattern

The protein bands of the UBHG were revealed to be between 6 kDa and 38 kDa, while the bands of the HBG were between 17 kDa and 98 kDa (Figure 4). Lobyaem (2005) reported that gelatin extracted from raw hide wastes using alkaline protease at 5,000 U/mg at 60°C for 3 h showed bands mainly between 18 kDa and 96 kDa. The presence of low molecular weight components in the UBHG indicates additional hydrolysis of collagen molecules from the liming process, as well as extraction at 70°C for 6 h. It also explains the lower values of gel strength and viscosity of the UBHG compared to those of the HBG (Table 6). Destruction of the covalent and the non-covalent bonds in gelatin, which stabilize the gelatin structure, due to extraction at high temperature and chemical pretreatment has been reported (Johnston-Banks, 1990; Tavernier, 1989; Zhao *et al.*, 2007).

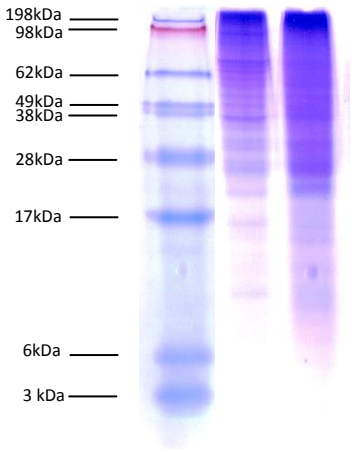

Figure 4 The SDS-PAGE patterns of (a) the standard protein marker, (b) the HBG, and (c) the UBHG.

3.2.6 Sorption isotherm

The sorption isotherm of the UBHG is as shown in Figure 5. The moisture content of the UBHG increased with the water activity (a_w). The curve indicates the adsorption process during equilibration. At a_w below 0.3, the gelatin was nonhygroscopic, as indicated by the flat curve. In contrast, at a_w above 0.3, the gelatin was hygroscopic, as indicated by the steep slope (deMan, 2013). The water activity range being between 0.35 and 0.45 was the beginning of changes in the physical state, including loss of crispness and stickiness of powders. These changes were controlled by the glass transition temperature (T_g), and it can be used to determine the critical moisture content where the changes commence (Labuza *et al.*, 2004).

Generally, the recommended water activity value of gelatin is less than 0.5 in order that the product is protected from growth of microorganisms (Pohlman, 2016). From this study, it is evident that the optimum relative humidity of 22% and temperature of 25°C can be used for protection of gelatin powder during storage.

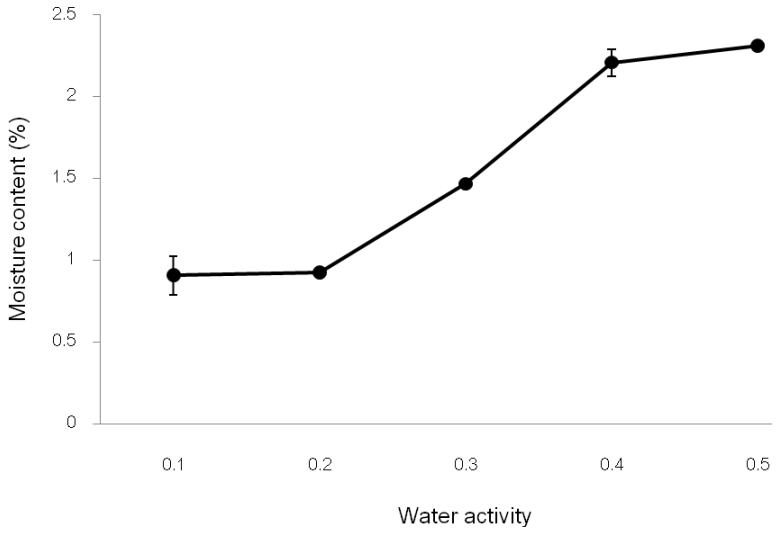

Figure 5 The sorption isotherm of the UBHG.

4. Conclusion

Underutilized bovine hide from the tannery industry could be successfully utilized for extracting crude gelatin. The use of acetic acid to extract gelatin from the tannery bovine hide could be a useful alternative to water treatment in that the extraction time could be considerably shortened, thus making it fit for industrial production. All the basic properties of the obtained gelatin were within the product standards, except color and turbidity which required further purification. The obtained low bloom gelatin is suitable for use in any application that requires gelatin with low gel strength.

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