

## Chemical composition and characterization of skin gelatin from buffalo (*Bubalus bubalis*)

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### Article history

Received: 16 January 2017

Received in revised form:

7 March 2017

Accepted: 8 March 2017

### Keywords

Buffalo's skin gelatin

Amino acid composition

Gel strength

Viscosity

Curing agents

### Abstract

About 90% gelatine is coming from porcine. Indonesia has largest muslim community worldwide, and muslim is prohibited to consume any products containing porcine gelatin. This study explores buffalo's skin gelatine and characterizes it based on physico-chemical properties. Gelatin from buffalo skin was extracted using different curing agents, namely alkaline of sodium hydroxide (NaOH) and neutral agent (the mixture of NaOH and citrate acid) with different molarity. The yield of gelatin using NaOH generally is higher than that of using the mixture of NaOH and citrate acid. FTIR spectra of gelatines revealed functional groups supporting the polypeptides chains. Amino acid profiles of gelatines extracted using different curing agents of NaOH 0.25 M; 0.50 M and 0.75 M have the similar profiles in terms of type and composition. Gelatin from buffalo's skin is potential to be developed as substitute of porcine gelatin.

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### Introduction

Gelatin, a fibrous protein prepared from hydrolysis of collagen, is an important polymer, extracted from skins, bones, and hides of mammalian animals. Currently, gelatin is also developed from fish sources (Karim and Bhat, 2009). Because of some differences in terms of collagen sources and preparation methods, gelatin presents a structure with variable physical properties and chemical heterogeneity (Djagny *et al.*, 2001). Gelatin is widely used in many types of application, such as in food and pharmaceutical products (Hidaka and Liu, 2003), in photography (ink jet printing), cosmetic and medical products (blood plasma substitutes, gelatin sponges) (Nhari *et al.*, 2012). Recently, its use expands to be functional food due to its ability to provide beneficial health effects (Jellouli *et al.*, 2011). The production of gelatin involves the hydrolysis of collagen either in acidic or alkaline environment produced from connective tissue of mammalian animals to meet the desired properties of gelatin (Arnesen and Gildberg, 2006).

The efficiency of gelatin extraction from bones and skin animals is affected by acidic or alkaline treatments. There are two types of gelatin, namely

type A and type B gelatines. Both types have different physicochemical and characteristics. For example, type A or acid treated collagen has isoelectric point at pH 6-9, and is appropriate for less fully cross-linked collagens commonly found in porcine or fish skin, while type B (an alkaline treated gelatin) reveals an isoelectric point at pH 5 (Benjakul *et al.*, 2009). Alkaline treatment is suitable for the more complex collagens found in bovine hides (Ahmad and Benjakul, 2011). Commercial gelatin was obtained from bovine and porcine, in which an approximately of 90% of gelatin is produced from porcine (Nhari *et al.*, 2012). Gelatin produced from cow can transmit the disease of bovine spongiform encephalopathy and foot and mouth disease. In addition, the Islamic religion prohibited its followers to consume any products containing porcine gelatin (Reegenstein *et al.*, 2003; Mursyidi, 2013). Therefore, some alternative gelatin coming from fish and halal animals such as bovine and other animals like buffalo must be developed. This study aims to explore buffalo's skin gelatine and to characterize it using several physico-chemical properties.

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## Materials and Methods

The skin of buffalo (*Bubalus bubalis*) was obtained from several slaughterhouses in Yogyakarta, Indonesia. The skin was cleaned and washed with iced tap water (0-2°C). Prepared skin was then cut into small pieces and stored at controlled room temperature until being used for preparation of gelatin.

### Preparation of gelatin

The gelatin extraction process of skin buffalo was Type B, according to Ockerman and Hansen (2000) with slight modification, using basic solution. Three curing solutions of 0.25 M; 0.50 M and 0.75 M NaOH were compared during gelatin extraction. In addition, the mixture of acid and basic solution, prepared as 0.25 M, 0.5 M and 0.75 M NaOH-citrate acid, respectively, was also investigated. The pretreated gelatin was placed into Beaker glass and was extracted in water bath at 55°C for 2 hour followed by boiling water for 5 h. The drying process of gelatin extracted was performed on conventional oven at 60°C for 3 days. Gelatin obtained was further subjected to characterization process.

### Determination of gel strength

Determination of Bloom gel strength was carried out according to Muyonga *et al.* (2004) using a texture analyser model TAXT2 (Stable Microsystem, UK). A solution containing 6.67% (w/v) gelatin was prepared by mixing 7.0 g of gelatin and 105 mL of distilled water in a Bloom bottle (150 mL). The mixture was maintained at 10°C and let to stand for 60 min in order to allow gelatin to absorb water and swell. The bottle was subsequently transferred to a water bath, maintained at 45°C and held for 30 min during which they were swirled intermittently. The samples were kept in refrigerator temperature for 17 h at 10°C before being subjected to gel strength determination. The Bloom gel strength (in g) was determined with the texture analyser set to make a 44 mm depression at a rate of 0.5 mm/minute. The gel strength (dyne/cm<sup>2</sup>) can be converted into Bloom using the equation of:

$$\text{Gel strength (dyne/cm}^2\text{) (D)} = \frac{F}{G} \times 980$$

$$\text{Bloom gel strength} = 20 + 2.86.10^{-3} D$$

F is graph height before fracture; G is a constant of 0.07 and D is gel strength in dyne/cm<sup>2</sup>.

### Determination of viscosity

Viscosity of gelatin was determined using Viscometer Brookfield RTV with spindle no 1 at 100 rpm, according to Arnesen dan Gildberg (2002). Gelatin sample used for Bloom gel strength determination was melted in a water bath maintained at 60°C for 15 min before the viscosity was determined. The measurement is initially performed at 60°C. The temperature is gradually lowered (0.25°C/ment) until gel is being formed. The viscosity can be seen in graph.

### Proximate analysis

The moisture and ash contents are determined using gravimetric method as in AOAC (1995). The fat contents were analysed using Soxhlet extraction using hexane as extracting solvent, while the protein content is determined using Kjeldahl method with conversion factor of 5.4. The carbohydrate level is obtained via residual analysis (Muyonga *et al.*, 2004).

### FTIR spectra measurement

FTIR spectra of gelatin is measured using FTIR spectrophotometer (Clair Scientific, Northampton, UK) equipped with a detector of deuterated triglycine sulphate (DTGS) and a beam splitter of KBr (Al-Saidi *et al.*, 2012). The gelatin samples were placed in attenuated total reflectance (ATR) crystal at room temperature (25°C). The spectrum were acquired in the region of 450-4000 cm<sup>-1</sup>. The spectra were recorded as absorbance values with 32 scanning at a resolution of 8 cm<sup>-1</sup> against air spectra as the reference. The spectrum were processed using Horizon MB software version 3.0.13.1 (ABB, Kanada).

### Amino acid composition

Amino acid composition of gelatin was determined using high performance liquid chromatography (HPLC) coupled with fluorescence detector (Lab Alliance<sup>TM</sup> 1200 Series) as in Widyaninggar *et al.* (2012). Ten gram of gelatin samples was accurately weighed and then subjected to autoclave for 24 hours with 10 mL HCl 6 N. Gelatine samples were neutralized with addition of NaOH or HCl 6 N. The Samples were transferred into 50-mL volumetric flask, added with 2.5 ml of Pb(II) acetate 40% w/v and 1 mL of oxalic acid 15% w/v. The samples were diluted until 50.0 with bidistilled sterile water to total volume of 50 mL and shaken smoothly. Furthermore, the sample was filtered using syringe-driven filter with pore size of 0.45 µm. Fifty microliter of filtered solution was added with 950 µL derivatizing reagents (orthophthalaldehyde-mercaptoethanol) and 30 µL solution was injected

Table 1. The values of pH, gel strength and viscosity of gelatine extracted from skin buffalo using different curing agent.

Curing agent	pH	Gel strength (Bloom)	Viscosity (cP)
0.25 M NaOH	6.69 ± 4.97	5.13 ± 1.56	4.00 ± 0.80
0.50 M NaOH	6.66 ± 4.94	4.01 ± 0.40	3.63 ± 0.56
0.75 M NaOH	10.26 ± 0.17	7.52 ± 4.59	4.05 ± 0.25
0.25 M NaOH -0.25 M citrate acid	7.16 ± 3.73	9.97 ± 6.80	4.29 ± 0.66
0.5 M NaOH -0.5 M citrate acid	10.02 ± 0.04	13.87 ± 7.37	4.19 ± 0.42
0.75 M NaOH -0.75 M citrate acid	4.34 ± 0.09	26.38 ± 4.41	3.97 ± 0.21

into HPLC system using Eurospher 100-5 C-18 (250 x 4.6 mm i.d., 5 μm). The mobile phase comprised of eluent A and eluent B, and is delivered in gradient manner. Eluent A is 0.01 M acetate buffer (pH 5.9), while eluent B is the mixture of 0.01 M methanol: acetate buffer (pH5.9): tetrahydrofuran (400: 75: 25 v/v/v).. The gradient program is minute 0-3, 30% eluent B; minute 3-25, 30%-100% B; minute 25.02 0% mobile phases B. The flow rate is 1.5 mL/min. The fluorescence detector was set at excitation and emission wavelengths at 340 and 450 nm, respectively.

**Results and Discussion**

The yield of buffalo’s skin gelatin recovered during extraction using optimized condition (NaOH 0.25 M; 0.5 M and 0.75 M) is about 13%. The lower yield obtained using basic curing agent is due to the fact that NaOH residue is bound in gelatin so that hydrolysis process is inhibited. The yield difference is also influenced by curing agent concentration, extraction method and raw materials used. Table 1 compiled pH values, gel strength and viscosity of gelatin extracted using different curing agents. Generally, pH values of gelatin extracted using NaOH are basic in nature, up to 10.26 ± 0.17, while that extracted using NaOH-citrate acid has pH almost neutral (pH 7).

The gel strength of gelatin affect its uses in food and pharmaceutical products. Gel strengthening during storage is mainly come from regeneration of helix structures among peptide chains in collagen and from hydrogen bonding formation between water molecules and hydroxylated amino acids (Haung et al., 2004; Arnesen and Gildberg, 2007). Table 1 compiled the gel strength (after storage 60 minute at 10°C) of buffalo’s skin gelatin extracted by different curing agents. The Bloom gel strength ranged from 4.01 ± 0.40 to 26.38 ± 4.41. These values are lower than Bloom strength of commercial bovine gelatin

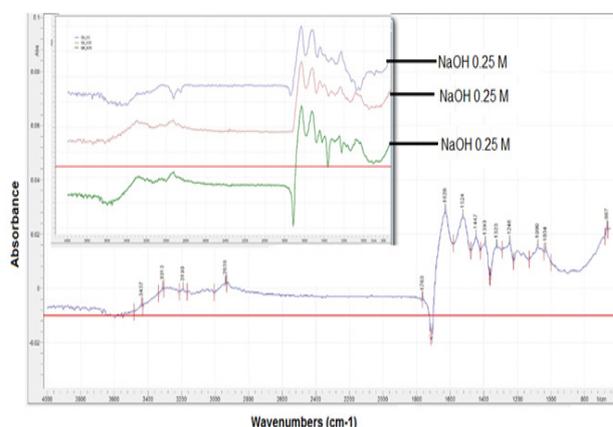


Figure 1. Typical FTIR spectra of gelatin from skin buffalo scanned at mid infrared region (4000-650 cm<sup>-1</sup>). Insert: FTIR spectra of gelatins extracted using NaOH 0.25, 0.5 and 0.75 M respectively.

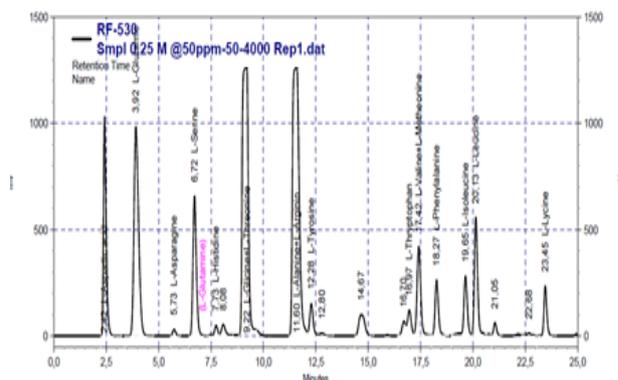


Figure 2. HPLC chromatogram of amino acids composition of buffalo’s skin gelatin extracted using 0.25 M NaOH.

(137.97 ± 1.17). The Bloom strength of skin buffalo is categorized as low gel strength (< 120), while commercial bovine gelatin is medium gel strength (120-200). There is no significant different of viscosity of gelatine extracted from different curing agents as shown in Table 1.

Figure 1 revealed FTIR spectra of gelatin from skin buffalo scanned at mid infrared region (4000-650 cm<sup>-1</sup>) which exhibited three prominent peak regions, namely at region 1 (3600-2700 cm<sup>-1</sup>) corresponding

Table 2. The proximate composition of gelatine extracted from skin buffalo using different curing agent.

Curing agent	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
0.25 M NaOH	8.78 ± 0.36	3.39 ± 0.03	0.41 ± 0.29	82.63 ± 0.21	4.79 ± 0.84
0.50 M NaOH	6.63 ± 0.06	3.21 ± 0.08	0.37 ± 0.01	83.21 ± 0.87	6.57 ± 0.73
0.75 M NaOH	8.87 ± 2.55	3.49 ± 0.85	0.75 ± 0.27	84.01 ± 1.46	2.88 ± 1.37
0.25 M NaOH - 0.25 M citrate acid	7.88 ± 1.34	4.05 ± 1.36	1.70 ± 1.20	NA	NA
0.5 M NaOH - 0.5 M citrate acid	9.32 ± 0.51	3.18 ± 0.24	0.78 ± 0.33	NA	NA
0.75 M NaOH - 0.75 M citrate acid	8.90 ± 0.72	2.83 ± 0.89	0.92 ± 0.04	68.59 ± 0.50	18.76 ± 0.63

Table 3. Amino acid composition of buffalo's skin gelatin extracted using NaOH as curing agents with different polarity.

Amino acid	Gelatine extracted using curing agent of		
	0.25 M NaOH (µg/g)	0.5 M NaOH (µg/g)	0.75 M NaOH (µg/g)
L-aspartic	2733.92	2662.71	2733.92
L-glutamic	4463.05	4371.37	4463.05
L-asparagine	94.55	88.92	94.55
L-histidine	503.20	491.05	503.20
L-serine	1657.82	1627.75	1657.82
L-glutamine	nd	nd	nd
L-tyrosine	703.51	614.41	703.51
L-phenylalanine	924.66	897.99	924.66
L-isoleucine	646.62	628.83	646.62
L-leucine	1399.81	1348.18	1399.81
L-lycine	2910.20	2987.14	2910.20
Thryptopan	375.91	6111.27	6094.43
L-valine + L-Methionine	1405.04	5916.61	5963.82
L-glycine + L-Threonine	6190.04	362.88	374.99
L-Alanine + L-arginine	6513.04	1314.54	1365.36

to Amide A and amide B, region 2 (1900-900  $\text{cm}^{-1}$ ) corresponding to Amide I, II and III, and region 3 (400-900  $\text{cm}^{-1}$ ) which related to Amide IV, V and VI (Pelton and McLean, 2000; Kong and Yu, 2007). All FTIR spectra have similar profiles in terms of number of peaks. The main functional groups responsible for IR absorption are N-H stretching (peptide bond) at 3400-3200  $\text{cm}^{-1}$ , C-H stretching at 3100-2800  $\text{cm}^{-1}$ , C=O stretching (peptide bond) at 1660-1600  $\text{cm}^{-1}$ , C-N-H bending at 1565-1500  $\text{cm}^{-1}$  and C-H bending at wavenumbers 1450-1300  $\text{cm}^{-1}$ . These functional groups support the polypeptides present in gelatin (Hermanto *et al.*, 2013).

HPLC at optimized condition can analyze amino acid composition in buffalo's skin gelatin with good separation among amino acids, unless L-valine co-eluted with L-Methionine, L-glycine with L-Threonine, and L-Alanine with L-arginine. An example of HPLC chromatogram obtained is shown in Figure 3. The composition of amino acids in buffalo's skin gelatin extracted using NaOH with different molarity is compiled in Table 3. It seems that buffalo's skin gelatin has the similar amino acid profiles in terms of amino acid type and amino acid composition.

## Conclusion

Gelatine extracted from buffalo's skin using NaOH with different molarity (0.25; 0.5; and 0.75 M) has the similar profiles in terms of pH, gel strength, viscosity, FTIR spectral profiles and amino acid composition with yield of about 13%. Gelatin from buffalo's skin is potential to be developed as halal gelatin to be used in food and pharmaceutical products.

## Acknowledgement

The authors thanks to the Ministry of Research and Higher education via research scheme Penelitian Unggulan Perguruan Tinggi 2016 awarded to Dr. Lily Arshanti with contract number: 782/UN1-P.III/LT/DIT-LIT/2016.

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