

## Effects of extraction conditions on characterization of gelatin from water buffalo (*Bubalus bubalis*) skin

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### Abstract

The study aimed to determine the characteristics of gelatin from water buffalo (*Bubalus bubalis*) skin pre-treated with NaOH and Ca(OH)<sub>2</sub> at different concentrations (0.3 M, 0.5 M and 0.7 M) and extracted at 65°C for 6 hrs and 24 hrs respectively. The gelatin obtained was evaluated for its moisture, protein and ash content, UV-vis absorption value, colour, emulsifying and foaming properties. The highest yield (20.25%) was observed for gelatin extracted by 0.5 M NaOH at 24 hrs extraction time. For alkaline pre-treatment, it was found that NaOH was more efficient than Ca(OH)<sub>2</sub> in terms of preparing the skin for subsequent extraction process. The protein content of the extracted gelatin samples was in the range of 71.76% - 87.83%, showing that the varying processing conditions are sufficiently to recover protein from the raw material. Ash content for all samples was in agreement with USDA standard, which was below than 3%. The extracted gelatin had varying pH values which were from 5.47 to 7.02. The gelatin was colourless with 'L' values of more than 80, except for 0.7 M Ca(OH)<sub>2</sub> at 24 hrs which showed slightly darker properties. The intensity of the UV-vis absorption spectrum showed that a high absorption peak was observed at 6 hrs of extraction time (230 – 250 nm) compared to 24 hrs extraction time. Emulsifying properties of buffalo gelatin increased with increasing concentrations of alkaline except for 0.7 M NaOH and 0.7 M Ca(OH)<sub>2</sub> for both extraction time. Meanwhile, foam expansion of the gelatin extracted from the different extraction conditions was observed to have a significant difference ( $p < 0.05$ ) for all samples. To our knowledge, buffalo skin has the potential to be an alternative source of gelatin in the diversified industrial application by modifying the extraction conditions in order to produce gelatin with desired quality.

## 1. Introduction

In recent years, the demand for gelatin production becomes increasing owing to its remarkable versatility. The global gelatin market is expected to grow at the highest rate from USD 2.6 billion in 2018 to USD 3.6 billion in 2023 with compound growth of 6.6% during the forecast period (Markets and Markets, 2020). This functional polymer has found many applications in various industries including stabilizer and emulsifier in food products, drug delivering agent and capsule in pharmaceutical products, as well as a thickening agent in cosmetic products. In order to understand the functionality of this polymer in different systems, numerous studies have been conducted on gelatin extraction and characterization from various sources.

Several studies have been published on the production of gelatin alternatives, including chicken deboner (Rafieian *et al.*, 2013), bovine lung (Roy *et al.*, 2017), Yak skin (Xu *et al.*, 2017) and cattle hides (Amertaning *et al.*, 2019).

The functionality of gelatin in various applications is determined by its physico-chemical properties. Whereby these properties depend greatly on the nature of source material and processing conditions. Over past decades, researchers have been focusing on alternative gelatin source due to restriction in some religions and safety issues with gelatin derived from mammals such as cows and pigs. While those from aquatic sources such as pangasius catfish skin (Mahmoodani *et al.*, 2014), unicorn leatherjacket skin (Hanjabam *et al.*, 2013) and

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hoki skins (Mohtar *et al.*, 2010) were considered to have promising qualities and good extractability. However there is still limitations in certain application as compared to their mammalian counterpart.

Therefore, this study is an attempt to discover the use of an underutilized group of local bovine, which is water buffalo (*Bubalus bubalis*) as a source of gelatin. This species is considered to have good adaptability to local ecological conditions and display excellent disease resistance (Nanda *et al.*, 2003). In Sabah, buffalo has been used as a source of red meat, mainly as frozen meat exportation. Large amount of the skins are often discarded as waste, and they are rich in collagen, the precursor of gelatin. To date, minimal study was found to evaluate the characteristics of gelatin derived from buffalo skin.

A study on various acid treatments on the extractability and properties of gelatin from buffalo hides has been carried out by Mulyani *et al.* (2017). Arsyanti *et al.* (2018) have reported on different alkaline pre-treatment on the yield and properties of buffalo hide gelatin. Study on the addition of pineapple rind at the different ratio on buffalo hide for gelatin extraction has been reported by Aprizal *et al.* (2019). It was found that gelatin of different characteristics can be obtained with varying treatment conditions. For instance, some showed high gel strength, some possessed low gel strength and certain conditions can produce a good yield of gelatin, whereas some showed poor yield. Therefore, it is of interest to deeply explore on the fundamental knowledge of processing conditions for gelatin derived from buffalo skin as a promising source for future industrial applications.

## 2. Materials and methods

### 2.1 Materials

Fresh water buffalo skin (*Bubalus bubalis*) (1kg) was supplied by Huswani Enterprise, Kota Kinabalu, Sabah. The skin was kept in freezer at -40°C prior to usage. Commercial papain enzyme (EC 3.4.33.3; 30000 USP-U/mg) was obtained from Merck (Darmstadt, Germany). All chemicals used were of analytical grade.

### 2.2 Gelatin extraction

Buffalo skin was washed and cut into a smaller size (1 cm x 1 cm). The skin was weighed to 100 g and was drip-dried. Extraction of gelatin from the skin of buffalo was done according to Mulyani *et al.* (2017) with slight modifications. Two different alkaline pre-treatments were carried out by using NaOH or Ca(OH)<sub>2</sub> at solution concentration of 0.3 M, 0.5 M and 0.7 M, respectively.

The skin was soaked in alkaline solution for 48 hrs at room temperature. Alkaline treated skin was then washed six times or more until the pH turned neutral. The skin was treated with 0.5 M acetic acid mixed with 10,000 units of papain for 24 hrs at room temperature, after which it was washed until neutral pH. This was followed by soaking in distilled water (1:4 w/v) at 65 °C for 6 hrs or 24 hrs, respectively. The mixture was then filtered through Whatman No. 1 filter paper and stored in a container at -42°C. The resultant filtrate was freeze-dried at -40 °C for three days and was used for further analyses.

### 2.3 Analyses

#### 2.3.1 Yield of gelatin

The amount of gelatin extracted was calculated using the formula as described by Xu *et al.* (2017).

$$\text{Yield} \left( \frac{\text{g}}{100} \right) = \frac{\text{Dry weight of gelatin (g)}}{\text{Wet weight of skin (g)}} \times 100$$

#### 2.3.2 Proximate composition

Determination of protein, moisture, ash, and lipid content were done according to AOAC (2000) method.

#### 2.3.3 UV-vis Spectra Absorption

UV spectra of the buffalo skin gelatin was evaluated using a UV-vis spectrophotometer (Perkin Elmer, Lambda 25, USA) according to the method performed by Xu *et al.* (2017). Samples (1 mg/mL) were dissolved in distilled water. Absorption curve of 190 to 400 nm with 0.5 nm resolution was used. Sample absorbance was recorded by using distilled water as standard.

#### 2.3.4 Colour properties

Colour was measured according to Mulyani *et al.* (2017). Dry gelatin was measured using a chromometer (Konica Minolta Sensing, Inc., Japan) using the Hunter system and expressed by brightness (L \*), redness (a \*), and yellowish (b \*).

#### 2.3.5 Emulsion properties

The emulsion activity index (EAI) and the emulsion stability index (ESI) of gelatin samples were determined by using the method as described by Roy *et al.* (2017). Six mL of gelatin solution (3% w/v) and 2 mL of corn oil was homogenized at 20,000 rpm for 1 min. The emulsions were then filtered out at 0 and 10 minutes and diluted with 0.1% of sodium dodecyl sulphate (SDS) solution for 100 times. The mixture was thoroughly mixed using a vortex mixer for 10 s. The resultant dispersion reading was measured using a spectrophotometer at 500 nm absorbance. EAI and ESI

are calculated as follows:

$$EAI(m^2/g) = (2 \times 2.303 \times A \times DF) / l \times \phi \times C \times 10,000$$

Where  $A = A_{500}$ ,  $DF =$  dilution factor (100),  $l =$  path length of cuvette (m),  $\phi =$  oil volume fraction and  $C =$  protein concentration in aqueous phase ( $g/m^3$ )

$$ESI (min) = \frac{A_0}{\Delta A} \times \Delta t$$

Where  $A_0 = A_{500}$  at time of 0 min;  $A_{10} = A_{500}$  at time of 10 min and  $\Delta t = 10$  min.

### 2.3.6 Foaming properties

The foam expansion (FE) and foam stability (FS) was determined using the method as described by Roy *et al.* (2017). The gelatin solution (3% w/v) was transferred to a 100 mL cylinder and homogenized at room temperature for one min at 13,400 rpm. The sample was then allowed to stand for 0, 30, and 60 mins. The FE and FS values were calculated as follows:

$$FE (\%) = (V_T/V_0)/V_0 \times 100$$

$$FS (\%) = (V_t/V_0)/V_0 \times 100$$

where  $V_T =$  total volume after whipping;  $V_0 =$  the original volume temperature before whipping and  $V_t =$  total volume after leaving at room temperature at different time.

### 2.4 Statistical analysis

All data obtained were analyze with analysis of variance (ANOVA) and differences between means were evaluated by Duncan's multiple range test (Steel and Torrie, 1980). For data analysis, the SPSS Statistic Program (Version 10.0) (SPSS, 1.2, 1998) was used.

## 3. Results and discussion

### 3.1 Yield

The yield of gelatin obtained from buffalo skin by different concentration of NaOH and  $Ca(OH)_2$  (0.3, 0.5 and 0.7 M, respectively) and different extraction time are as shown in Table 1. In general, the use of NaOH in alkaline pre-treatment and prolonged extraction time was observed to increase the yield of gelatin (Table 1). The highest yield was obtained for gelatin extracted by 0.5 M NaOH at 24 hrs extraction time. NaOH is seen to be more efficient than  $Ca(OH)_2$  in gelatin extraction as observed in Table 1, where during 6 hrs extraction time NaOH at concentration of 0.5 M was able to give the same yield value (no significant different at  $p < 0.05$ ) with higher concentration of  $Ca(OH)_2$  (0.7 M).

In the present study, the gelatin extraction time was carried out for 6 hrs, in accordance to Mulyani *et al.*

(2017). However, the duration was observed to be insufficient for gelatin extraction. As shown in Table 1, the concentration of NaOH solution will increase the yield percentage at 6 hrs extraction time. Whereas the yield was observed to drop after a certain concentration (0.5 M) at 24 hrs extraction time with NaOH pre-treatment. These were explained that over hydrolysis of gelatin has occurred. The findings are corroborated with findings reported by Roy *et al.* (2017) which bovine lung gelatin was extracted for 24 hrs where the yield reduced after 24 hrs. Karim and Bhat (2009) suggested that prolonged extraction time will further destroy the hydrogen bond by breaking down the covalent cross-links in the sample, thus lowering the rate of gelatin production.

Table 1. Gelatin yield with different extraction time and alkaline pre-treatment concentrations.

Extraction Time (hr)	Alkaline Concentrations (M)	Yield (%)
6	NaOH 0.3	4.40±0.44 <sup>a</sup>
	NaOH 0.5	7.52±0.53 <sup>b</sup>
	NaOH 0.7	9.52±0.35 <sup>c</sup>
	$Ca(OH)_2$ 0.3	2.69±0.38 <sup>d</sup>
	$Ca(OH)_2$ 0.5	5.71±0.26 <sup>c</sup>
	$Ca(OH)_2$ 0.7	8.27±0.34 <sup>bf</sup>
24	NaOH 0.3	13.15±0.44 <sup>g</sup>
	NaOH 0.5	20.25±0.34 <sup>h</sup>
	NaOH 0.7	17.64±0.07 <sup>i</sup>
	$Ca(OH)_2$ 0.3	10.79±0.18 <sup>j</sup>
	$Ca(OH)_2$ 0.5	14.84±0.16 <sup>k</sup>
	$Ca(OH)_2$ 0.7	17.66±0.29 <sup>il</sup>

Results are expressed as mean±SD (n = 3). Different superscript letters in the same column indicate significant difference ( $p < 0.05$ ).

NaOH and  $Ca(OH)_2$  are the two most frequently used alkali in the process of gelatin extraction. Both of these alkalis have the same function of removing non-collagen proteins. According to Zhou and Regenstein (2005), the alkali used at the same pH value showed that NaOH was able to extract almost twice as much non-collagen protein from cow skin at 4°C as compared to  $Ca(OH)_2$  irrespective the effect when protease was added. Since NaOH is a strong alkaline, thus causing a significant swelling to the skins during pre-treatment, whereas  $Ca(OH)_2$  which is a weak alkaline did not perform that property (Liu *et al.*, 2015). Moreover, according to Liu *et al.* (2015), NaOH solution is the most widely used in gelatin extraction as it provides more open collagen structure to the skin which facilitates the transfer rate of the solvent into the intercellular substance.

Table 2. Proximate analysis of gelatin obtained from buffalo skin pre-treated with different concentrations of alkaline and extraction time

Extraction Time (hr)	Alkaline Concentrations (M)	Protein	Ash	Moisture	Fat		
6	NaOH	0.3	78.18±0.48 <sup>a</sup>	1.62±0.48 <sup>abfjl</sup>	4.48±0.08 <sup>a</sup>	7.63±0.05 <sup>a</sup>	
		0.5	83.41±0.63 <sup>b</sup>	1.15±0.10 <sup>abcfghijkl</sup>	3.28±0.26 <sup>b</sup>	5.65±0.08 <sup>b</sup>	
		0.7	80.55±0.40 <sup>c</sup>	0.80±0.31 <sup>bcjkl</sup>	6.52±0.43 <sup>c</sup>	3.45±0.12 <sup>c</sup>	
	Ca(OH) <sub>2</sub>	0.3	71.76±0.91 <sup>d</sup>	2.59±0.26 <sup>de</sup>	6.04±0.80 <sup>cd</sup>	7.31±0.03 <sup>ad</sup>	
		0.5	77.67±1.00 <sup>ac</sup>	2.62±0.13 <sup>e</sup>	7.59±0.27 <sup>ce</sup>	5.35±0.13 <sup>bc</sup>	
		0.7	87.83±0.28 <sup>f</sup>	1.54±0.19 <sup>abfjkl</sup>	8.10±0.22 <sup>efk</sup>	3.27±0.12 <sup>cf</sup>	
	24	NaOH	0.3	83.12±0.09 <sup>bg</sup>	0.57±0.29 <sup>bcghijkl</sup>	1.55±0.07 <sup>g</sup>	8.81±0.20 <sup>g</sup>
			0.5	86.15±0.19 <sup>h</sup>	0.44±0.32 <sup>bcghijk</sup>	4.93±0.13 <sup>afh</sup>	4.49±0.29 <sup>h</sup>
			0.7	72.02±0.40 <sup>di</sup>	0.16±0.04 <sup>cgghik</sup>	3.03±0.23 <sup>bi</sup>	1.17±0.12 <sup>i</sup>
Ca(OH) <sub>2</sub>		0.3	87.10±0.50 <sup>fhjhh</sup>	1.05±0.08 <sup>abcfghijkl</sup>	8.12±0.34 <sup>efjk</sup>	8.22±0.21 <sup>j</sup>	
		0.5	84.43±0.16 <sup>bk</sup>	0.81±0.14 <sup>bcfghijkl</sup>	8.72±0.04 <sup>bfjk</sup>	4.51±0.24 <sup>hk</sup>	
		0.7	82.39±0.42 <sup>bgl</sup>	1.24±0.78 <sup>abcfjgk</sup>	1.03±0.12 <sup>gl</sup>	1.04±0.11 <sup>il</sup>	

Results are expressed as mean±SD (n = 3). Different superscript letters in the same column indicate significant difference (p < 0.05).

### 3.2 Proximate composition

Proximate composition of gelatin derived from buffalo skin as affected by different extraction conditions are as shown in Table 2. The maximum protein content for the 6 hrs extraction time was generated by Ca(OH)<sub>2</sub> at a concentration of 0.7 M (87.83%). While at 24 hrs of extraction time, the maximum protein content was produced by 0.3 M Ca(OH)<sub>2</sub> (87.10%). The results were almost consistent with other studies such as Mulyani *et al.* (2017) that stated the protein content for buffalo skin was within 83.38±0.06% and 91.11±0.03%. Furthermore, present studies showed similar pattern to the commercial gelatin protein values of 89.63% (Pranoto *et al.*, 2007).

Based on the Table 2, the ash content for each parameter ranges from 0.16% to 2.59%. This value meets the GMIA standard (2019), which ranges from 0.3% to 2.0%. The maximum ash content of 2.6% has been suggested by Mulyani *et al.* (2017) and Muyonga *et al.* (2004). Low ash content is an indicator of a good quality of gelatin extraction process (Muyonga *et al.*, 2004; Uriarte-Montoya *et al.*, 2011; Mulyani *et al.*, 2017).

From this study, it can be observed that the alkaline solution of NaOH is better used to remove minerals in buffalo skin compared to the alkaline solution of Ca(OH)<sub>2</sub>. This is due to the ash content of gelatin produced using 0.3 M NaOH was similar to that of gelatin produced with 0.7 M Ca(OH)<sub>2</sub> for the same extraction time of 6 hrs. According to Mulyani *et al.* (2017), the use of strong alkaline solution during the pre-

treatment process can result in high levels of gelatin produced, but the gelatin has low functional properties. This may be due to the alkali were used to break the collagen bond (Schmidt *et al.*, 2016) and cause excessive hydrolysis which resulted gelatin with a small molecular weight dispersion (Rodrigo, 2009).

The moisture content of the buffalo skin gelatin was in the range of 1.03 to 8.72%. Based on Gomez-Guillen *et al.* (2002) studies, extracted gelatin must contain less than 15% moisture content. The results of the moisture content studies using buffalo skin are in line with this requirement because the moisture content for all parameters is less than 15%. As for fat, the results indicate that high concentration of alkaline is better in removing fat in the buffalo skin as the fat content 0.7 M of NaOH and Ca(OH)<sub>2</sub> for both 6 and 24 hrs extraction are at the lowest.

### 3.3 pH

The pH value is essential in determining the gel strength of gelatin as the gel strength is determined by the gelatin isoelectric point and this isoelectric point can be controlled by adjusting the pH (Gudmundsson and Hafsteinsson, 1997). As stated by Arsyanti *et al.* (2018), type B gelatin has isoelectric at pH 5. The pH of extracted gelatins in the present study varied from 5.47 to 7.02, which were higher than 5 coherent to the collagen was neutralized using alkali, which may have raised its pH values.

### 3.4 Colour

The colour properties of buffalo skin gelatin were

shown in Table 3. The  $L^*$  value for all samples are more than 80 except for the sample that treated with 0.7 M NaOH at 24 hrs extraction time that has a value of 72.32, hence confirming the visual observations on the gelatins. Generally, the gelatin was yellowish which explained by the Maillard reaction induced by the free amino acid released by the acid in contact with the C = O component found in the gelatin (Jridi *et al.*, 2014; Mulyani *et al.*, 2017). The colour properties are different between gelatin and this may be due to the colour extraction of the skin of the raw material itself (Jamilah *et al.*, 2011). Colour has aesthetic value as it does not in theory that influences the functional properties of gelatin, but only used to satisfy the consumer needs (Zarai *et al.*, 2012; Shyni *et al.*, 2014; Mulyani *et al.*, 2017).

### 3.5 UV-vis

The absorption of UV-vis spectra for buffalo skin gelatin using different extraction times and different concentrations of NaOH or Ca(OH)<sub>2</sub> were described in the Table 3. The results showed that the spectrum characters of the buffalo skin gelatin in the range of wavelength 210 to 250 nm. Extraction time at 6 hrs showed a high absorption rate for NaOH and Ca(OH)<sub>2</sub> at all concentrations ranging from 230 to 260 nm, indicating the presence of a peptide bond in the gelatin polypeptide chain (Xu *et al.*, 2017). The result is corroborated with past studies by Hermanto *et al.* (2013) reporting that UV-vis spectra of bovine gelatin type B were in the range of 210 to 240 nm. A high absorption peak (4.3035) was observed for gelatin samples treated with 0.5 M Ca(OH)<sub>2</sub> at 6 h of extraction time compared to other samples which attributable to C=O changed

from  $\rho$  to  $\pi$  due to peptide linkages (Xu *et al.*, 2017). Meanwhile, lower absorbance was observed at 24 hrs extraction time which might be due to low molecular weight of gelatin subsequently because of further hydrolysis (Xu *et al.*, 2017). There is a little absorption region at 220 – 240 nm for all gelatin samples was observed due to excess non-aromatic components such as phenylalanine, tyrosine and tryptophan (Xu *et al.*, 2017).

### 3.6 Emulsion property

The EAI and ESI of the buffalo skin gelatin are presented in Table 4. The maximum value for EAI was observed at 0.5 M Ca(OH)<sub>2</sub> for 6 hrs extraction time (67.03 m<sup>2</sup>/g) while the maximum value for ESI was at 0.3 NaOH for 6 hrs extraction time (98.33 min). There is significance difference (p<0.05) in general for gelatin samples treated at 6 hrs extraction time for both NaOH and Ca(OH)<sub>2</sub> and the values decreasing as the concentration of alkaline solution increasing. According to Xu *et al.* (2017), lower value of EAI and ESI are characterized by low dispersion of gelatin molecular weight. From the present study, it can be seen that all parameters exhibited good emulsion properties as the values are much higher than Yak skin gelatin which ranging from 7.14 m<sup>2</sup>/g to 37.58 m<sup>2</sup>/g for EAI and 20.99 min to 41.46 mins for ESI (Xu *et al.*, 2017). Thus it can be concluded that the uses of alkali with high concentration results in a weaker emulsion and the use of NaOH has observed to be better than Ca(OH)<sub>2</sub> to produce gelatin with good emulsion properties.

Table 3. Physicochemical properties buffalo skin extracted using different concentrations of alkaline and extraction time.

Extraction Time (hr)	Alkaline Concentrations (M)	$L^*$	$a^*$	$b^*$	pH	UV-vis	
						Maximum Absorption	Absorbance (nm)
6	NaOH						
	0.3	81.28±0.42 <sup>a</sup>	3.06±0.03 <sup>aij</sup>	3.74±0.37 <sup>aj</sup>	6.82±0.01 <sup>a</sup>	4.1478	240
	0.5	88.16±0.16 <sup>befjk</sup>	2.59±0.01 <sup>bh</sup>	7.37±0.01 <sup>bh</sup>	5.79±0.01 <sup>b</sup>	3.9871	250
	0.7	90.28±0.54 <sup>ce</sup>	3.21±0.02 <sup>cdi</sup>	2.19±0.04 <sup>cdfig</sup>	6.11±0.04 <sup>c</sup>	4.0258	250
	Ca(OH) <sub>2</sub>						
	0.3	86.01±0.40 <sup>dgl</sup>	3.20±0.12 <sup>acdi</sup>	2.27±0.40 <sup>cdefg</sup>	7.02±0.02 <sup>d</sup>	4.1333	230
0.5	89.24±0.17 <sup>befj</sup>	3.57±0.02 <sup>e</sup>	1.38±0.07 <sup>cde</sup>	5.86±0.02 <sup>bc</sup>	4.3035	250	
0.7	88.23±0.28 <sup>befjk</sup>	3.18±0.04 <sup>acdfgi</sup>	2.48±0.12 <sup>cdefgi</sup>	6.04±0.01 <sup>ef</sup>	4.0194	250	
24	NaOH						
	0.3	85.67±0.44 <sup>dgl</sup>	3.28±0.05 <sup>cdghi</sup>	2.36±0.34 <sup>cdfig</sup>	6.07±0.04 <sup>cfg</sup>	3.7682	250
	0.5	84.09±0.47 <sup>h</sup>	2.68±0.05 <sup>bhijk</sup>	7.26±0.38 <sup>bh</sup>	5.47±0.12 <sup>h</sup>	3.9034	210
	0.7	72.32±0.58 <sup>i</sup>	3.19±0.08 <sup>adfgij</sup>	14.17±0.20 <sup>i</sup>	6.40±0.02 <sup>i</sup>	3.6924	250
	Ca(OH) <sub>2</sub>						
	0.3	87.83±0.95 <sup>befjk</sup>	2.99±0.00 <sup>acj</sup>	3.34±0.07 <sup>afj</sup>	5.97±0.01 <sup>efgj</sup>	3.7236	250
0.5	88.79±0.63 <sup>befjk</sup>	2.76±0.04 <sup>hk</sup>	5.67±0.16 <sup>k</sup>	5.70±0.03 <sup>bk</sup>	3.8987	210	
0.7	85.72±0.63 <sup>dgl</sup>	2.62±0.02 <sup>bhkl</sup>	9.82±0.80 <sup>l</sup>	6.39±0.02 <sup>il</sup>	3.9099	220	

Results are expressed as mean±SD (n = 3). Different superscript letters in the same column indicate significant difference (p < 0.05).

Table 4. Functional properties of buffalo skin extracted using different concentrations of alkaline and extraction time.

Extraction Time (hr)	Alkaline Concentrations (M)	Emulsion Activity Index (m <sup>2</sup> /g)	Emulsion Stability Index (min)	Foaming Expansion (%)	Foaming Stability (%)	
					(30 mins)	(60 mins)
6	NaOH					
	0.3	52.31±0.14 <sup>a</sup>	98.33±0.31 <sup>a</sup>	9.53±0.07 <sup>a</sup>	4.34±0.12 <sup>a</sup>	4.34±0.12 <sup>a</sup>
	0.5	58.60±0.30 <sup>b</sup>	82.24±0.48 <sup>b</sup>	14.03±0.08 <sup>b</sup>	9.50±0.12 <sup>b</sup>	8.98±0.85 <sup>b</sup>
	0.7	25.45±3.91 <sup>cg</sup>	70.38±0.23 <sup>c</sup>	23.60±0.20 <sup>c</sup>	13.97±0.03 <sup>c</sup>	13.23±1.06 <sup>c</sup>
	Ca(OH) <sub>2</sub>					
	0.3	36.55±0.33 <sup>d</sup>	87.87±0.50 <sup>d</sup>	18.58±0.21 <sup>d</sup>	7.46±0.35 <sup>d</sup>	7.07±0.21 <sup>d</sup>
24	0.5	67.03±0.12 <sup>e</sup>	28.30±0.37 <sup>c</sup>	30.13±0.12 <sup>c</sup>	21.87±0.16 <sup>c</sup>	19.24±0.45 <sup>c</sup>
	0.7	23.18±0.11 <sup>cfg</sup>	25.43±0.49 <sup>f</sup>	20.05±0.11 <sup>f</sup>	18.02±0.11 <sup>f</sup>	16.03±0.16 <sup>f</sup>
	NaOH					
	0.3	59.08±0.05 <sup>bg</sup>	84.03±0.16 <sup>g</sup>	21.27±0.30 <sup>g</sup>	17.41±0.42 <sup>fg</sup>	14.82±0.24 <sup>fg</sup>
	0.5	49.99±0.08 <sup>ah</sup>	88.39±0.86 <sup>dh</sup>	19.93±0.13 <sup>fh</sup>	10.93±0.19 <sup>sh</sup>	10.95±0.22 <sup>h</sup>
	0.7	46.93±0.12 <sup>hi</sup>	56.11±0.26 <sup>i</sup>	20.59±0.16 <sup>fi</sup>	10.41±0.39 <sup>i</sup>	10.19±0.19 <sup>bhi</sup>
	Ca(OH) <sub>2</sub>					
	0.3	65.16±0.14 <sup>efgi</sup>	71.68±0.25 <sup>j</sup>	24.67±0.19 <sup>j</sup>	12.99±0.12 <sup>j</sup>	5.92±0.21 <sup>dj</sup>
	0.5	55.03±0.13 <sup>bk</sup>	95.57±0.20 <sup>k</sup>	19.04±0.23 <sup>dk</sup>	0.99±0.24 <sup>k</sup>	0.99±0.24 <sup>k</sup>
	0.7	49.46±0.34 <sup>bijl</sup>	20.48±0.29 <sup>cjl</sup>	20.15±0.38 <sup>fl</sup>	0.85±0.18 <sup>kl</sup>	0.57±0.29 <sup>kl</sup>

Results are expressed as mean±SD (n = 3). Different superscript letters in the same column indicate significant difference (p < 0.05).

### 3.7 Foaming

The FE and FS at 30 mins and 60 mins are shown in Table 4. In general, all of these experimental parameters showed good foam stability as the maximum value of FE (30.13%) and FS (30 mins = 21.87% , 60 mins = 19.24%) of buffalo skin are similar to Yak skin gelatin (FE= 35.6% , FS at 30 mins = 19.54% , FS at 60 mins = 18.52%). According to Xu *et al.* (2017) this indicates that buffalo skin gelatin has a broad molecular weight distribution with higher surface viscosities and lower steric stabilization. Interestingly, the use of NaOH solution showed better foam stability compared to Ca(OH)<sub>2</sub> for both extraction times. The stability of the foam increases with increasing alkali strength which suggests that alkaline pre-treatment using NaOH solution is preferable to produce a large molecular weight dispersion gelatin, thus having the desired foam stability compared to Ca(OH)<sub>2</sub>.

## 4. Conclusion

Gelatin extracted from water buffalo's skin using 0.5 NaOH at 24 hrs of extraction time was observed to be the best extraction conditions for producing the highest gelatin yield. Moreover, the resulting gelatin also showed satisfactory physicochemical and functional properties; hence water buffalo skin has the potential to be an alternative source of gelatin for industrial application.

### Conflict of interest

The authors declare no conflict of interest.

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