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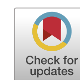
Original article

Extraction and characterization of gelatin from camel skin (potential halal gelatin) and production of gelatin nanoparticles



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ABSTRACT

Gelatin is used as an ingredient in both food and non-food industries as a gelling agent, stabilizer, thickener, emulsifier, and film former. Porcine skins, bovine hides, and cattle bones are the most common sources of gelatin. However, mammalian gelatins are rejected by some consumers due to social, cultural, religious, or health-related concerns. In the present study, gelatin was obtained from camel skin as an alternative source using a combination of processing steps. Central composite design combined with response surface methodology was used to achieve high gelatin yields under different extraction conditions: temperatures of 40, 60, and 80 °C; pH values of 1, 4, and 7; and extraction times of 0.5, 2.0, and 3.5 min. Maximum gelatin yield from camel skin (29.1%) was achieved at 71.87 °C and pH 5.26 after 2.58 min. The extracted gelatin samples were characterized for amino acid profile, foaming capacity, film formation, foam stability, and gel strength (Bloom value). Gelatin nanoparticles were produced, and their morphology and zeta potential were determined. Bloom value of the camel skin gelatin was 340 g. Amino acid analysis revealed that the extracted gelatin showed high glycine and proline contents. Analysis of camel skin gelatin nanoparticle and functional properties revealed high suitability for food and non-food applications, with potential use in the growing global halal food market.

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1. Introduction

Gelatin is a protein product originally derived from the partial hydrolysis of collagen obtained from skin (hides), bones, and connective tissues of land animals, usually mammals, as well as of fish and chickens. Gelatin is commonly used as an ingredient for enhancing elasticity, thickness, and emulsification (Lin et al., 2017). Physical properties of gelatin, as measured by Bloom value and viscosity, are the most important. The Bloom value gives a measure of gelatin strength, classified as low (≤150 g), medium (>150–220 g), and high (>220–300 g) Bloom (AL-Kahtani et al., 2017).

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The halal status of mammalian gelatin is controversial and sometimes considered haram. Halal is the term referring to some animal-based products that must be produced under specific criteria according to sharia "Islamic legislation". Pork and its by-products are not allowed to be consumed according to Islamic teaching. Consumption (as food or in pharmaceutical products) and use (non-food products such as cosmetics) of Haram (non-halal) products are prohibited (Regenstein et al., 2003). However, meat and its derivatives from other ruminants including cows, goats, sheep, and camels can be consumed, although only when the animal is slaughtered according to Islamic teaching (Fuseini et al., 2016). In addition, diseases such as bovine spongiform encephalopathy (BSE) in cows and swine flu in pigs raise safety concerns (Herpandi et al., 2011).

Interest in alternative sources of halal gelatin is increasing due to growing concerns among industry and consumers. The growing demand for halal gelatin in halal foods, and the rejection of haram sources of gelatin (mainly porcine gelatin) have encouraged scientists to search for alternative sources. Fish gelatin has been widely studied in the past decade by many institutions worldwide.

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Gómez-Estaca et al. (2009) reported that both warm- and cold-water fish species represent an alternative source of gelatin that would be permitted under some religious practices (halal and kosher) and would likely also be disease-free. However, fish gelatin is of poorer quality, with lower Bloom value and yield (Gómez-Estaca et al., 2009), than the superior mammalian gelatins. Therefore, a new mammalian gelatin source would be desirable. Potentially, camels could be an interesting novel source of gelatin. To date, no studies have been conducted on extraction and characterization of gelatin from camel skin. Therefore, the present study focused on extraction optimization and characterization of gelatin from camel skin using central composite design combined with response surface methodology (CCD-RSM) for applications in nanotechnology.

2. Material and methods

2.1. Raw materials and pretreatment

Hides of healthy camels sacrificed at a slaughter house in Riyadh, Saudi Arabia, were procured. Soft tissues, periosteum, and muscles were removed. Next, the skins were salted and stored in airtight containers. The salted skins were de-haired by soaking in an alkaline solution of either NaOH + sodium sulfide (SS) or lime + SS for 3 days at 10 °C and then cut into pieces (Fig. 2A). The de-haired camel skin was then thoroughly rinsed with water to remove the solution (Fig. 1).

2.2. Camel skin proximate composition

Proximate composition of the camel skin, including moisture, ash, and fat, was determined according to the methods of the American Association for Cereal Chemistry (AACC, 2010).

2.3. Gelatin extraction optimization

Distilled water (1:3 skin:water w/v) was added to pretreated camel skin, and gelatin was extracted according to the design layout provided by CCD-RSM 6.0.8 (Minneapolis, USA), followed by filtration and freeze drying (Fig. 1). The weight of extracted gelatin was calculated as g dry gelatin/100 g of camel skin.

2.4. Camel skin gelatin nanoparticles

2.4.1. Preparation of nanoparticles

Camel gelatin nanoparticles were prepared according to the method described by Coester et al (2000) with some modifications. Gelatin (1.25 g) from camel skin was dissolved in 25 ml water under constant stirring and heating (40 °C). Desolvation and rapid sedimentation of gelatin were achieved by the addition of 25 ml acetone. The sediment was redissolved in 25 ml water under heating, and the solution pH was adjusted to 3.

Gelatin was dissolved again by dropwise addition of 40 ml acetone. After 10 min of stirring, 100 μl glutaraldehyde (25%, w/w) was added to crosslink the particles. After stirring for another 12 h, the dispersion was centrifuged at 12,000*g* for 15 min. The particles were purified by three-fold centrifugation and redispersion in acetone (30%). After the last redispersion, acetone was evaporated in a water bath at 50 °C. The particles were then stored at −4 °C.

2.4.2. Morphology

Morphology of the dried gelatin samples was determined using a dual-beam scanning electron microscope (SEM; FEI Quanta 3D FEG, FEI Ltd., Hillsboro, USA) equipped with a silicon drift detector.

2.4.3. Zeta potential

Zeta potential was measured (in triplicate) using Zetasizer Nano ZS (Malvern Instruments, UK) at pH 2, 4, and 6 and 50–200 kilo counts/s.

2.5. Amino acid profile

Between 0.1 and 0.2 g of camel skin, bovine, and porcine (Sigma-Aldrich) gelatin was hydrolyzed with 5 ml 6 N hydrochloric acid at 110 °C for 24 h (Nemati et al., 2004). Amino acids were analyzed using Waters high-performance liquid chromatography system (Model 2695, Massachusetts, USA) with AccQ Tag column (3.9 × 150 mm) at 36 °C and an injection volume of 5 μl. AccQ Tag eluent A concentrate and 60% acetonitrile were filtered using a 0.45-μm regenerated cellulose membrane. The flow rate was set at 1 ml/min. Data were acquired and analyzed using a fluorescence detector (Model 2475, Milford, Massachusetts, USA) and processed using Waters Empower Pro software.

2.6. Foaming properties

Camel skin gelatin solution (25 ml) was sonicated using a Vibracell ultrasonic processor (VCX-750, Sonics Inc., Newtown, Connecticut, USA) at frequency of 20 kHz and amplitude of 95% (wave amplitude of 108 mm at 100% amplitude) for 2 min. After 15 min the foaming stability was measured. Foam formation capacity and foam stability were calculated according to the following equations:

Foam formation capacity = Volume of foam at 0 s/Solution initial volume

Foam stability = Volume of foam at 15 min/Volume of foam at 0 min.

2.7. Gel strength (Bloom Value)

Camel skin gelatin strength (Bloom value) was determined according to the method described by Boran and Regenstein (2010). Gelatin solution at a concentration of 6.67% (w/v) was prepared in a flask (2.3 × 3.6 cm) with distilled water. The Bloom value was measured using a texture analyzer (Stable Micro Systems, Surrey, England) with a 30-kg load cell and flat-faced cylindrical plunger with a diameter of 1.27 cm. When the plunger had penetrated 4 mm into the gel surface, the force maximum in grams was recorded.

2.8. Film formation

Camel skin gelatin solution for film formation was prepared using distilled water at a concentration of 4 g/100 ml. Plasticizers (sorbitol and glycerol, 0.15 g/g gelatin) were added, and the mixture was homogenized with warming and stirring at 40 °C for 15 min. Aliquots of 40 ml were then used to cast films in square dishes (12 × 12 cm) followed by drying in a conventional oven at 45 °C for 15 h. Films were conditioned for 2 days at 58% relative humidity over an NaBr-saturated solution in a desiccator at 22 °C, after which the film thickness was measured.

3. Results and discussion

3.1. Camel skin proximate composition

Proximate composition of camel skin before and after the de-hairing is presented in Table 1. Fat content of the de-haired sample reduced to 4.75% (from 6.11%) but moisture and ash content increased (from 12.51% to 74.69% and from 0.73% to 1.47%,

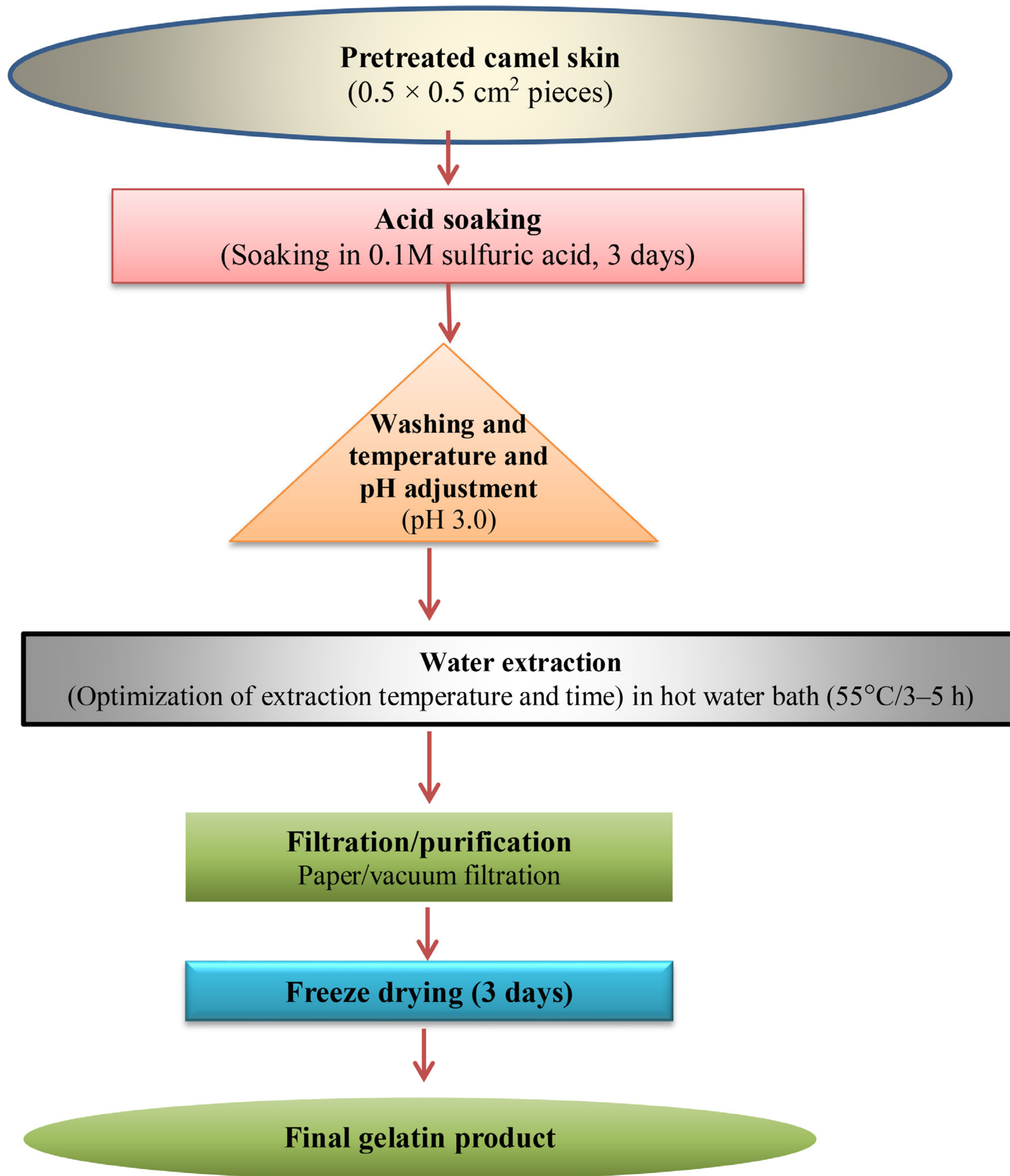

Fig. 1. Camel skin gelatin extraction.

Table 1
Proximate composition and physical characteristics of camel skin gelatin.

Sample	Moisture (%)	Fat (%)	Ash (%)	Bloom (g)	Film thickness (cm)	Foam (%)
Raw skin	12.51	6.11	0.73	–	–	–
De-haired skin	74.69	4.75	1.47	–	–	–
Gelatin	–	–	–	340.15 ± 0.5	0.5	10

respectively). These results are consistent with the long soaking time required in the de-hairing solutions, although there are no available data in the literature for comparison.

3.2. Camel skin gelatin extraction optimization

Gelatin yield ranged from 9.57% to 28.59% (Table 2). Statistical analysis (result not shown) revealed that extraction time significantly ($p < 0.05$) affected camel skin gelatin extraction. This pronounced impact of extraction time could be due to the additional time required to uncoil collagen crosslinks before gelatin formation. Coefficient models for camel skin gelatin yield were created using regression analysis. The developed models were relevant ($p < 0.05$) and suitable for predicting gelatin yield. The following equation was used:

Table 2
Optimization results: Yield* of gelatin from camel skin using CCD-RSM.

Temperature (°C)	pH	Time (min)	Skin gelatin yield (%)
80	7	0.5	9.57
60	4	0.5	11.11
60	4	2.0	28.59
80	4	2.0	24.93
80	1	0.5	9.22
60	4	2.0	28.59
40	4	2.0	13.54
40	1	3.5	25.86
60	4	2.0	28.59
60	4	3.5	25.73
80	7	3.5	27.98
60	7	2.0	14.94
60	4	2.0	28.59
60	4	2.0	28.59
40	1	0.5	10.71
40	7	3.5	27.22
80	1	3.5	16.45
60	4	2.0	28.59
60	1	2.0	27.30
40	7	0.5	9.74

*The highest yield of gelatin (29.1%) was produced at 71.87 °C and pH 5.26 after 3.2 min.

$$\text{Gelatin yield of camel skin} = +26.34 + 0.11A - 8.738E - 003B + 7.29C - 3.72A^2 - 1.83B^2 - 4.54C^2 + 1.44AB - 0.88AC + 1.69C \quad (1)$$

where A = temperature, B = pH, and C = extraction time

A graphical format (Fig. 2B) was adopted to more easily visualize optimum conditions. Within the range of extraction conditions used, optimization was achieved at certain points. Further statistical analysis indicated optimal yield of 29.1% gelatin from camel skin at 71.87 °C and pH 5.26 after 2.58 min.

3.3. Camel skin gelatin nanoparticles

3.3.1. Morphology

SEM showed that polystyrene (PS-20)-based skin gelatin nanoparticles were rectangular and tended to aggregate irregularly, while sodium caseinate-based nanoparticles were more bead-like and tightly packed in a regular fashion (Fig. 3A). Similar findings have been reported for gelatin porosity and morphology (Barbetta et al., 2005).

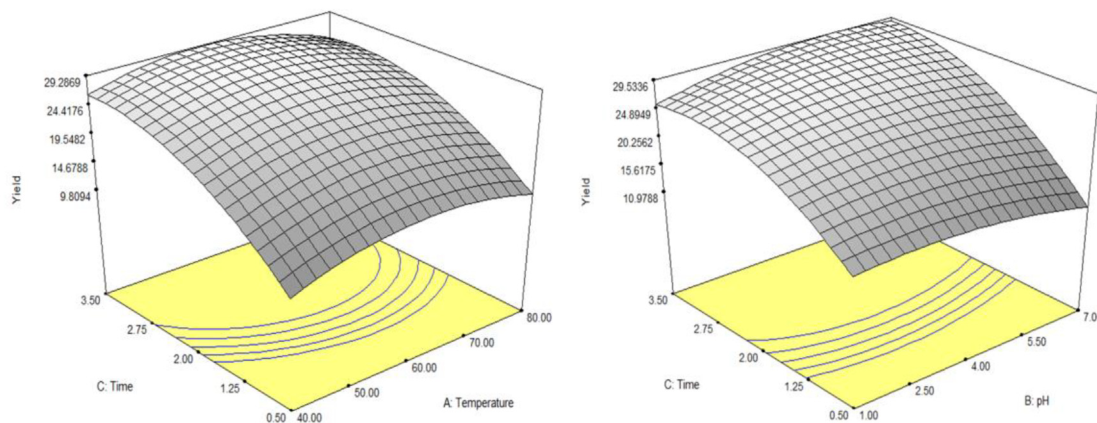

Fig. 2. (A) Camel skin after hair removal. (B) Three-dimensional plots of extraction optimization of gelatin yield (%).

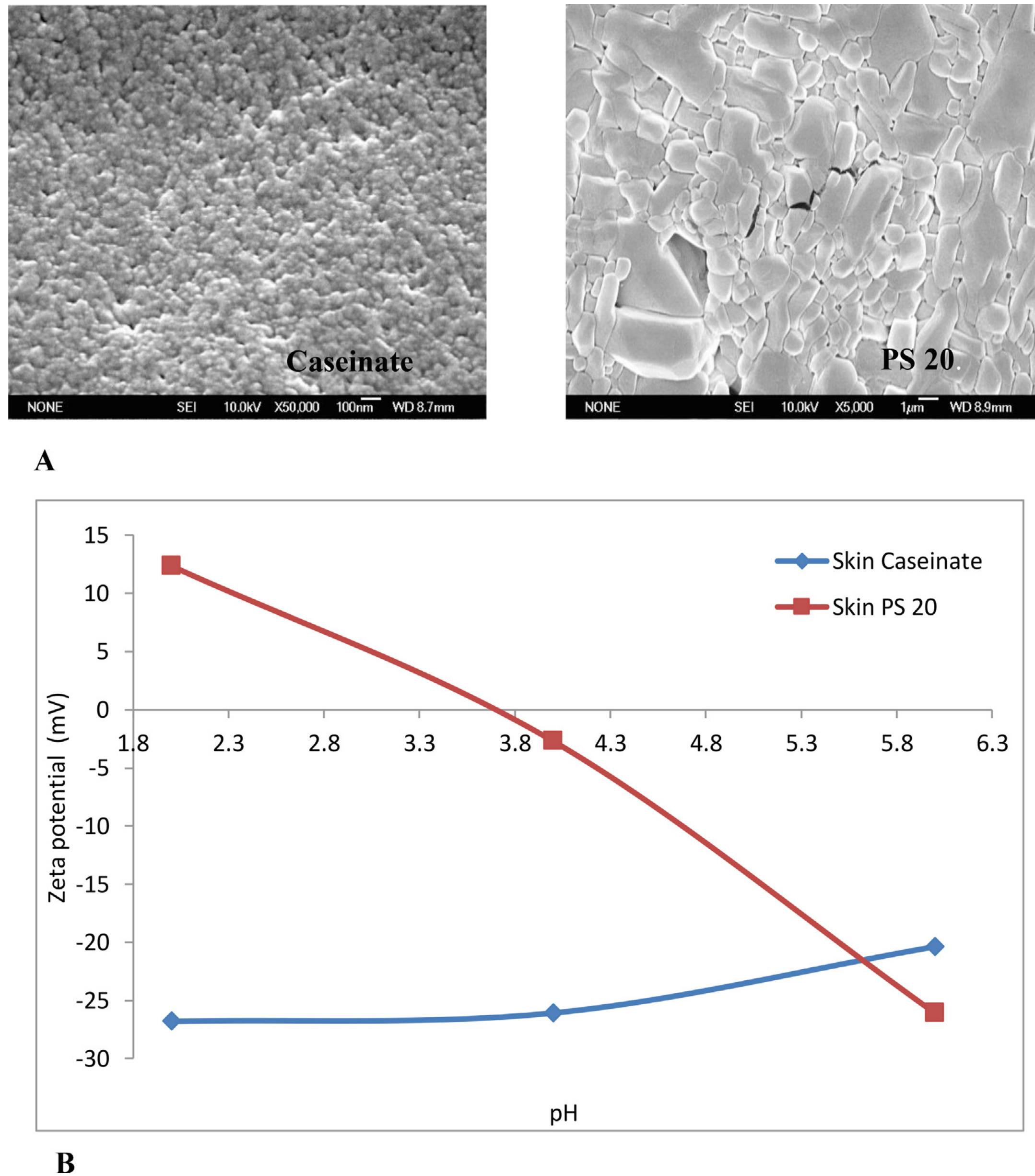

Fig. 3. (A) Scanning electron micrographs showing camel skin gelatin nanoparticle morphology. (B) Zeta potentials plot for polystyrene-based (PS-20) and casein-based nanoparticles at different pH.

3.3.2. Zeta potential

The surface charge on gelatin nanoparticles is referred to as the zeta potential, and it reflects their long-term stability. Being made of protein, stability of gelatin nanoparticles is a function of pH of the surrounding conditions. The zeta potential of all gelatin nanoparticles was stable at pH 2 and 6. At pH 4, only the sodium caseinate-based gelatin nanoparticles were stable, where conditions were close to their isoelectric point (Fig. 3B). Similar results were observed during desolvation in a study of gelatin surface charge by Ahsan and Rao (2017).

3.4. Camel skin gelatin amino acid profile

Regarding proteins in general, the functional properties of camel skin gelatin were influenced by their amino acid profile. Camel skin gelatin showed high glycine, proline, and lysine

Table 3
Amino acid profiles of camel skin, bovine, and porcine gelatin (mg/100 g).

Amino acid	Camel skin	Bovine	Porcine
Aspartic acid	25	17	41
Glutamic acid	52	34	63
Serine	14	12	21
Glycine	101	108	109
Histidine	3	2	3
Arginine	17	47	41
Threonine	15	15	35
Alanine	15	33	80
Proline	57	63	151
Tyrosine	4	10	8
Valine	13	10	14
Methionine	4	4	10
Isoleucine	20	7	12
Leucine	11	12	29
Phenylalanine	17	10	27
Lysine	37	11	27

contents (Table 3) similar to bovine and porcine gelatins. High glycine content generally presents as glycine at every third position in the polypeptide chain (nearly one-third of its residues). Previous reviews on the quality of biofilm properties of gelatin have highlighted important roles of imino acids (proline and hydroxyproline) and glycine. Increased resistance to gelatin film deformation is due to high content of proline and its role in collagen structure. The secondary collagen structure arises from the presence of imino acids (proline and hydroxyproline) that stabilize the triple helix and prevent rotation of the polypeptide backbone (Nogrady and Weaver, 2005; Schrieber and Gareis, 2007). Lysine content was higher in camel skin gelatin than in porcine and bovine gelatin.

3.5. Foaming capacity and foam stability

In addition to its gelling behavior, gelatin shows desirable foaming properties by increasing the viscosity of the aqueous phase and thus reducing surface tension at the liquid–air interface (Gómez-Guillén et al., 2011). The foaming capacity of camel bone gelatin (10%) is higher than that of camel skin gelatin (4%) (Table 1).

Foam stability is principally dependent on film nature and reflects interactions among proteins in the foam matrix (Aewsiri et al., 2011). Foam stability of camel bone gelatin (4%) was higher than that of camel skin gelatin (2%) (AL-Kahtani et al., 2017). Generally, foaming capacity and foam stability of proteins are associated with their hydrophobic properties. Increase in the number of hydrophobic protein groups may significantly improve surface properties, including foaming capacity and foam stability (Toledano and Magdassi, 1998).

3.6. Bloom value (gel strength)

Bloom value of camel skin gelatin was 340 ± 0.5 g (Table 1). This value is similar to that of porcine skin gelatin (350.4 g), higher than that of bovine skin gelatin (266.69 g), and exceeds the "high" Bloom category (Bloom value, 100–300 g) of mammalian gelatin. Gelatins with a Bloom value of 50–260 g are in commercial demand; make-up artists use gelatin with a Bloom value of 300 g for special effects (to create fake wounds, for example), while the beverage industry uses gelatins with a low Bloom value to clear cloudiness (Schrieber and Gareis, 2007).

3.7. Film formation

Thickness of camel skin gelatin film was 0.5 cm (Table 1), which is somewhat less than that of bovine (0.8 cm) and porcine (0.9 cm) skin gelatin. Thickness of camel skin gelatin films was within the range reported for bovine and tuna skin gelatin films and was consistent with previously reported values. Gelatin film improves the resistance of foodstuffs (e.g., fruits, meat, and so on) against exposure to light, oxygen, and drying (Gómez-Guillén et al., 2011; Mariod and Adam, 2013).

4. Conclusion

Gelatin was obtained from camel skin using CCD-RSM to optimize extraction conditions (temperature, pH, and time), and the extracted gelatin was successfully characterized. Camel hide could be a valuable source of gelatin and provide an alternative to porcine gelatin, which is of particular significance in the halal market.

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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