Physicochemical Properties of Peking Duck Skin Gelatin Extracted Using Acid Pretreatment (ADS) or Mixed Alkaline-Acid Pretreatment (ALDS)

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Abstract. Duck skin is the by-product of duck meat production, and it is a readily available source of gelatin that may serve as an alternative to gelatin made from pigs and cows. In this study, the physicochemical properties of Peking duck skin gelatin were assessed. Duck skin gelatin was extracted using acid pretreatment (ADS) or mixed alkaline-acid pretreatment (ALDS). The extraction yield of ALDS (1.95%) was significantly higher than that of ADS (1.33%), and the recovery of protein of ALDS was 46.47% compared to 43.77% for ADS. The bloom value of ADS (364.10 g) was significantly higher than that of ALDS (205.13 g) and commercial type B bovine gelatin (BG, 224.20 g). The high bloom value of ADS and medium bloom value of ALDS mean that they can be used in many food applications. The hydroxyproline content of ADS (13.84 g/100 g) also was significantly higher than that of ALDS (10.25 g/100 g) and BG (12.87 g/100 g). The pH of ADS and BG (5.31 and 4.90, respectively) did not differ significantly, whereas the pH of ALDS was 8.34. Viscosity values of ADS and ADLS were 13.51 and 12.35 mPas, respectively, which were significantly higher than that of BG (3.62 mPas). Overall, these results show that duck skin is a potential raw material for gelatin production, as it has a high bloom value and is readily available in Malaysia.

1 Introduction

Collagen derived from skin, white connective tissue, and bones of animals undergoes partial hydrolysis to produce gelatin. Gelatin is a high molecular weight polymer, and most of its function derives from the collagen triple helix, which is the basis of the polypeptide gel network [1]. Gelatin can be produced in two ways: type A gelatin undergoes acid pretreatment, and type B gelatin undergo alkaline pretreatment.

In the industrial setting, gelatin is produced by processing the waste generated during animal slaughtering. Bovine and porcine sources are common and provide good quality gelatin [2]. However, ethical, religious, environmental, and safety considerations have led to the quest for gelatin replacers, such as polysaccharides or polymers [3]. In particular, the emergence of bovine spongiform encephalopathy in the 1980s made the search for gelatin replacers a major issue in the world today [4].

Thirty percent of the waste from fish processing consists of skin and bone, which has high collagen content and is an excellent material for producing gelatin. However, fish-derived gelatin is less stable and has weak rheological properties compared to gelatin derived from land mammals [5]. Another

alternative gelatin source is poultry skin, which is a waste product of the poultry processing industry. The chemical composition of chicken-derived gelatin is similar to that of bovine gelatin, and it has better physicochemical properties compared to fish gelatins [6].

Duck production has increased in last two decades. Ref [7] and Ref. [8] reported the potential used of duck meat as an ingredient in processed poultry products, meanwhile Ref. [9] and Ref. [10] reported the potential extraction of collagen and gelatin from of duck feet. Gelatin extracted from duck skin is also a potential ingredient for use in functional foods [11]. After China and France, Malaysia is the third largest producer and exporter of duck meat. Therefore, it could supply sufficient amounts of duck skin as the raw material for industrial-scale gelatin production [12].

The objectives of this study were to evaluate the physicochemical properties of gelatin extracted from Peking duck skin and to compare these properties between gelatins extracted using acid pretreatment (ADS) and mixed alkaline-acid pretreatment (ALDS). Properties also were compared to those of commercial type B bovine gelatin (BG).

2 Materials and Methods

2.1 Source of duck skin

Duck skin used in this study was from the Peking duck species. Duck skin was obtained from Perak Duck Food Industries Sdn Bhd, Taiping, Perak, Malaysia. All duck skin was stored below –18 °C in a freezer. These products were Halal certified by the Islamic Development Department of Malaysia. Acetic acid and sodium hydroxide were used in the gelatin extraction process, and alcohol 1-butanol was used to remove fat from the duck skin.

2.2 Extraction of gelatin

Gelatin was extracted according to Lee et al. [11] with slight modification. Duck skin was thawed at 5 °C for 24 hours. Thawed skin was cut into small pieces, and the fat was removed prior to gelatin extraction as follows. Duck skin was homogenized with alcohol solution using a blender (Panasonic MX-799, Japan), and it then was suspended in 10% (v/v) alcohol solution at 1:8 ratio of duck skin/solution at 5–7 °C for 48 hours. The alcohol solution was changed every 24 hours by pouring off the solution and replacing it with new alcohol solution at the same ratio 10% (v/v).

Defatted duck skin was pretreated in two different ways: ALDS and ADS. After the pretreatment, the duck skin was washed for 2 hours until neutral pH was obtained. Gelatin was extracted by water at 65 °C for 2 hours at a ratio duck skin/distilled water ratio of 1:1.

2.3 Acid pretreatment (ADS)

The acid pretreatment process followed Lee et al. [11] with slight modification. Defatted duck skin was treated with 0.1 M acetic acid at a sample/solution ratio of 1:7 for 40 minutes with gentle stirring. This step was repeated three times with changing of the acetic acid solution. Duck skin then was washed under running tap water for 2 hours to reach neutral pH. Gelatin was extracted by water and freeze dried to obtain a dry gelatin.

2.4 Mixed alkaline-acid pretreatment (ALDS)

The mixed-alkaline acid pretreatment procedure also followed Lee et al. [11] with slight modification. This pretreatment can weaken the collagen structure and at the same time solubilize the non-collagen proteins and hydrolyse the peptide bonds to maintain the consistency of collagen fibers [13]. Defatted duck skin was subjected to 2% sodium hydroxide solution at a sample/solution ratio of 1:7 for 40 minutes with gentle stirring. This step was repeated three times with changing of the sodium hydroxidesolution. Duck skin then was subjected to 0.7% citric acid solution at a sample/solution ratio of 1:7 for 40 minutes with gentle stirring, followed by washing under running tap water for 1 hour to reach neutral pH. Gelatin then was extracted by water and freeze dried to obtain a dry gelatin.

2.5 Gelatin extraction yield

Gelatin extraction yield was calculated based on the wet weight of the raw material as follows:

$$= \frac{\text{weight of dried gelatin powder (g)}}{\text{wet weight of raw defatted duck skin (g)}} \times 100\ \%$$

2.6 Protein recovery

Protein recovery value is obtained according to Boran and Regenstein [14] by comparing the amount of protein extracted gelatin with the amount of protein in raw duck skin. The purpose of protein recovery is calculated to evaluate the efficiency of the extraction process relative to the starting material.

$$= \frac{\text{Protein content of gelatin extracted}}{\text{Protein content of raw duck skin}} \times 100\ \%$$

2.7 Proximate analysis

The moisture, ash, protein, and fat contents of the samples were measured using AOAC [15] methods. Protein was determined by the Kjeldahl method and fat content was measured by the Soxhlet method.

2.8 Hydroxyproline content

Hydroxyproline content was measured following AOAC [15] methods. Duck skin was cut into small pieces before proceeding to the hydrolysis step. For the analysis, 2 g of sample were weighed into a test tube, which was covered before 15 ml of sulphuric acid were added into it. The test tube was placed in a drying oven at 105 °C ± 1 °C for 16 hours. The hot hydrosylate was transferred to a 500 ml volumetric flask. The volume was diluted with water and mixed. Next, 5 ml of the solution were filtered into a 100 ml volumetric flask to ensure that the hydroxyproline concentration of the final dilution was in the range of 0.5 to 2.4 µg/ml. Next, 2.0 ml of the final dilution were pipetted into a test tube, and 2.0 ml of distilled water were added to two additional test tubes to act as blanks. Then, 1.0 ml of oxidant solution was added to each test tube. The mixture was shaken and then left standing for 20 minutes ± 2 minutes at room temperature. After 20 minutes, 1.0 ml of colour reagent was added, and the solution was mixed thoroughly. Each test tube was capped with a screw cap, and the body of the test tube was covered with aluminum foil. The test tubes were immediately placed in a water bath at 60 °C ± 0.5 °C for exactly 15 minutes, followed by cooling under running tap water for at least 3 minutes. The tubes were dried, and absorbance of solutions versus blanks was measured in 10 mm glass cells at 558 ± 2 nm with a Shimadzu UV-160 Uv-vis spectrophotometer. Standard solutions were prepared from stock solution (6 µg/ml) at 0.6, 1.2, 1.8, and 2.4 µg hydroxyproline/ml. Hydroxyproline content was calculated according to the following formula:

2.9 Bloom value (Gel strength)

The bloom value of gelatin gels was determined according to the British Standards Institute method [16]. Gelatin gel was prepared by dissolving 6.67% (w/v) gelatin powder in distilled water at room temperature; it was left to solubilize for 30 minutes. The mixture was heated in a water bath at 60 °C for approximately 15 minutes until the gelatin was completely dissolved. The gelatin solution was cooled to room temperature and stored at 10.0 °C ± 0.5 °C for 18 hours prior to analysis. A TA TX2 texture analyzer (Stable Microsystems, Surrey, UK) was used to determine the gel strength with a 5 kg load cell and a standard radius cylinder (P/0.5) probe. Bloom value (g) was obtained when the probe penetrated into the gel to a depth of 4 mm. The maximum force reading, which is the resistance to penetration, was obtained and translated into bloom strength (g) of the gel.

2.10 Colour

Colour of the gels was measured using a colorimeter. The tristimulus L*a*b* measurement mode was used determine colour. L* represents lightness (L* = 0 for black, L* = 100 for white), a* represents red/green (+a* is red and –a* is green), and b* represents blue/yellow (+b* is yellow intensity and –b* is blue intensity). The gelatin gel sample was prepared and poured into a Petri dish, in which readings were taken.

2.11 Viscosity

Viscosity of the gelatin was determined according to Norziah et al. [17]. First, 6.67% gelatin solution was prepared and allowed to equilibrate at 60 °C for 30 minutes. Viscosity then was measured using a Sine-wave Vibro viscometer (SV-10, A&D Co., Ltd, Japan).

2.12 pH

The British Standards Institute method BSI [16] was used to determine the pH of the 1.0% gelatin solution. Gelatin powder was dissolved in distilled water for 30 minutes, heated to 60 °C for 30–60 minutes, and then cooled before pH was measured using a pH meter (Metter Toledo, S20 Seveneasy, USA).

2.13 Statistical analysis

All analyses were performed in duplicate experiments, with triplicate analysis for each experiment. The data were analyzed by analysis of variance using SPSS version 16.0. Statistical significance was defined at $p < 0.05$.

3 Result and Discussion

Table 1 shows the extraction yield and protein recovery of the ADS and ALDS pretreatment methods. The gelatin extraction yield of ALDS was significantly higher than that of ADS. However, both values were lower than that of chicken skin gelatin extracted using alkaline and acid pretreatment (2.16) [6]. The extraction yield was low because the calculation is based on wet weight of raw duck skin. The gelatin extraction yield from chicken deboner residue (10.2) was much higher than that of the ADS and ALDS results for duck skin gelatin. The gelatin extraction yield of chicken feet skins and tendons (7.83) was higher than those of chicken feet paws (7.37), metatarsus (5.97), and integers (6.30) [18]. Fish skin gelatin from cod (14) [19], red tilapia (7.81), black tilapia (5.39) [20], sin croaker (14.3), and shortfin scad (7.25) [21] also had higher gelatin extraction yield compared to the duck skin gelatin in this study.

Table 1. Extraction yield and protein recovery of the ADS (Acid) and ALDS (Alkaline) pretreatment methods

Sample	Extraction yield	Protein recovery
ADS	1.33 ± 0.12^{b}	43.77 ± 0.42^{b}
ALDS	1.95 ± 0.12^{a}	46.47 ± 0.55^{a}

[abc]Values are means of three replicates ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

The purpose of alkaline treatment is to destroy chemical cross-linkages that are still present in the collagen that will give stock its firmness and also to remove any unwanted material or impurities from collagenous material [13]. Sin croaker skins that were subjected to alkaline and acid pretreatment tended to swell more, possibly because the cross-links were open during swelling and thus contributed to the higher extraction yield. This might explain why ALDS had higher extraction yield than ADS [21]. Ref. [22] reported that the yield of gelatin slightly increased with increasing extraction time. Longer extraction time provides more energy to destroy bonds and free the α- or β-chains from the skin complex. However, Ref. [23] reported that extraction times longer than 6 hours did not increase the extraction yield significantly. A low yield of gelatin can be caused by leaching of collagen during washing and treatment of skin. Insufficient denaturation of soluble collagen during extraction also can contribute to a low gelatin yield [24].

In this study, the protein recovery for ALDS was significantly higher than that of ADS (Table 1). Boran and Regenstein [14] reported the highest protein recovery of gelatin from silver carp skin to be 78.1%. Stroma proteins are abundant in poultry skin, and the main content of stroma protein is collagen [25]. Collagen content in cow skin protein is 90 to 95% [26]. Mammalian tissue used as raw material for gelatin extraction consists of collagen, elastin, and mucopolysaccharides, which are sources of organic and inorganic impurities. Other proteins from muscle, blood, keratins, glycoproteins, and mucopolysaccharides, as well as hyaluronic acid, keratasulphate, chondroitin, sulphate, lipids, nucleic acids, and other cell components are present in tissues, and these components probably contribute to the small amounts of soluble degradation products present in gelatin. The purpose of pretreatment is to solubilize or disperse some of these constituents so that they are removed during subsequent washing. Extraction conditions are selected to specifically solubilize gelatin, and impurities are left behind in the insoluble residue or as finer particles, which can be removed by filtration [27]. Table 2 shows the chemical composition of raw duck skin, ADS gelatin, ALDS gelatin, and commercial BG. Gelatins that are extracted with high protein and low ash and fat contents indicate an efficient gelatin extraction process [28].

Table 2. Chemical composition of ADS (Acid), ALDS (Alkaline), and BG (Bovine)

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
Raw duck skin	–	18.66 ± 1.42	–	–
ADS	7.58 ± 0.59^{a}	81.59 ± 0.74^{c}	5.41 ± 0.18^{a}	0.84 ± 0.11^{b}
ALDS	4.48 ± 0.71^{b}	86.69 ± 1.04^{b}	5.92 ± 0.31^{a}	2.89 ± 0.04^{a}
BG	2.86 ± 0.29^{b}	89.85 ± 1.41^{a}	1.57 ± 0.18^{b}	1.58 ±0.54^{b}

^{abc}Values are means of three replicates ± standard deviation.
Different letters in the same column indicate significant differences ($p < 0.05$).

The moisture content of ADS was significantly higher ($p < 0.05$) than that of ALDS and BG. Moisture contents of gelatin extracted from chicken skin (9.81%) [6] and chicken feet and tendons (10.39%) [18] were higher than the ADS, ALDS, and BG values in this study. Fish gelatin from horse mackerel skin (12.1%) [29], tiger toothed croaker skin (9.60%), and pink perch skin (8.73%) [24] also had higher moister content than ADS, ALDS, and BG. In contrast, gelatin from black kingfish skin (6.04%) [30] and cuttlefish skin (6.48%) [31] had lower moisture content than ADS. Moisture contents of ADS, ALDS, and BG were all within the range of 8.0% to 12.0%. Water vapor-tight packaging is recommended, as gelatin can absorb or release moisture depending on the humidity of the surrounding air. There is a risk of lump formation and microbiological growth if the moisture content exceeds 16% [32].

The protein content of BG was significantly higher than that of ADS and ALDS (Table 2). In comparison, chicken skin gelatin (80.76%) [6] and chicken feet and tendons (84.96%) [18] also had high protein content. Skin gelatin from cuttlefish (91.35) [31], black kingfish (88.72) [30], tiger toothed croaker (86.45), pink perch (72.63) [24] had high protein content as well. Because the Kjeldahl method was used for protein determination of ADS, ALDS, and BG in this study, a conversion factor of 5.55 was used instead of 6.25, which is usually used. The conversion factor is used to count all of the nitrogen in the sample, making the result only an approximation of the actual protein present.

The fat content of ALDS and ADS did not differ significantly different (Table 2). In contrast, the fat content of cuttlefish skin (0.28%) [31] and black kingfish (0.48%) was much lower (i.e., almost fat free) [30], which showed that the extraction process had eliminated fats as desired [33]. The high fat content of ADS and ALDS (> 5%) indicated that the extraction method used did not eliminate fats as desired.

The ash content of ALDS was significantly higher than that of ADS and BG (Table 2). Chicken skin gelatin (0.37%) [6] and chicken feet and tendons (1.91%) [18] also had low ash content. The ash contents of horse mackerel skin gelatin (0.25%) [29], cuttlefish skin gelatin (0.05%) [31], tiger toothed croaker skin gelatin (1.88%), pink perch skin gelatin (0.30%) [24], ADS, and BG all were lower than the maximum limit of 2.6% as recommended [34]. In contrast, the ALDS ash content was > 2.6%, which indicates that it is not viable for use as a food ingredient. Further studies of the extraction of ALDS are needed to reduce the ash content.

The protein contents of raw duck skin (18.66%) (Table 2) and chicken skin (19.25%) [35] were higher than that of black kingfish skin (12.94%) [30], tiger-toothed croaker skin (17.50%), pink perch skin (16.76%) [24], and cuttlefish skin (14.00%) [31]. This comparison shows that the raw material from poultry skin has higher protein content than fish skin.

ADS had a significantly higher bloom value than ALDS and BG (Table 3). Chicken skin gelatin (355 g) [6] and chicken feet and tendons (294.78 g) both are other poultry sources of gelatin, and both had high bloom values. Fish skin gelatin from sole (350 g), yellowfin tuna (426 g) [36], and megrim (340 g) had high bloom values as well [5]. Horse mackerel skin gelatin (230 g) [29], black kingfish skin gelatin (222 g) [30], and cobia skin gelatin (232 g) [37] had medium bloom values. In contrast, bloom values of skin gelatin from tiger toothed croaker (170 g), pink perch (140 g) [24], cod (~ 90 g), hake (~110 g) [5] and cuttlefish (181 g) [31] were low. Gels made from fish gelatin with low bloom value can be used in non-gelling gelatins, refrigerated products, and products for which low gelling temperature is required [38]. The variation of bloom values observed among gelatins can be due to intrinsic characteristics such as protein chain composition, molecular weight distribution, amino acid content, type of extraction, and properties of collagen.

Table 3. Bloom value and hydroxyproline content of ADS (Acid), ALDS (Alkaline), and BG (Bovine)

Sample	Bloom value (g)	Hydroxyproline (g/100 g)
ADS	364.10 ± 3.10^{a}	13.84 ± 0.35^{a}
ALDS	205.13 ± 1.46^{b}	10.25 ± 0.37^{b}
BG	224.20 ± 3.03^{c}	12.87 ± 0.08^{c}

^{abc}Values are means of three replicates ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

A gelatin's ability to form hydrogen bonds with water molecules enables the formation of a stable three-dimensional gel. To evaluate the characteristics of gels, the concept of gel strength, which is also known as bloom value, was born [24]. When a hot gelatin liquid is cooled, the viscosity increases progressively, and the liquid gradually turns into a gel if the gelatin concentration is high and the temperature is low. Gelatin is special because of its ability to transform liquid into a solid form and retain its shape and elastic properties over a wide range of pH values. Gelatin does not require the addition of specific ions or other chemicals to aid in gelation [39]. The quality of gelatin is determined by the bloom value, which is characterized as low (< 150 g), medium (150–220 g), and high (220–300 g) [40].

The gelatin maturation process at low temperature for long periods of time is important, as it affects the bloom value as well [5]. Ref. [41] reported that gel strength mainly depends on the proportion of fractions with molecular weight of approximately 100,000 gmol–1, and there is a strong correlation between gel strength and α-chains. Thus, gelatins with higher α-chain content have greater gel strength. Gelatins with lower percentage of β- and γ-components compared to those with α-chains require a longer maturation time to allow for growth of the existing nucleation sites [5]. Ref. [4] reported that the ability of α-chains to anneal correctly may be reduced due to the presence of protein degradation fragments, which would hinder the growth of the existing nucleation sites. The inability of α-chains to anneal correctly leads to a lower bloom value. The origin of the raw material also influences the bloom value. Ionic strength is also thought to affect bloom value and deionization, thus pH adjustment from 4.5 to

about 6.0 can result in higher bloom value for fish gelatin [29]. A high bloom value also may contribute to high melting and gelling points and shorter gelling time of the final product[6].

The hydroxyproline content of ADS was significantly higher than that of ALDS and BG (Table 3). Ref. [33] reported that gelatins with high levels of imino acids tend to have a higher gel strength and melting point. Thus, ADS had a higher bloom value than ALDS and BG. Fish skin gelatins produced from black kingfish (8.34 g/100 g) [30], tiger toothed croaker (7.77 g/100 g), and pink perch (7.63 g/100g) [24] had lower hydroxyproline content than ADS, ALDS, and BG. The lower content of hydroxyproline and proline present in fish gelatins resulted in low gel modulus and gelling and melting temperatures [42]. Skin gelatin from cobia, which is a warm-water fish, had a higher hydroxyproline content (9.62 g/100 g) than croaker (8.77 g/100 g), which is a cold-water fish. A lower hydroxyprolinecontent was reported for gelatin extracted at temperatures of 70 °C and 80 °C, which resulted in lower gel strength compared to gelatin extracted at temperatures of 50 °C and 60 °C [28].

Ref. [11] studied gelatin hydrosylate produced from duck skin, and the amino acid analysis showed that the glycine content was highest for both alkaline pretreated gelatin (26.24%) and acid pretreated gelatin (26.04%), followed by hydroxyproline in alkaline pretreated gelatin (12.94%) and acid pretreated gelatin (12.78%). Relatively high contents of alanine, arginine, glutamic acid, and proline also were found in both gelatins. Gelatin contains all 20 amino acids, but their relative contributions differ among gelatins derived from different sources [41]. Mammalian protein contains large amounts of hydroxyproline, hydroxylysine, and total imino acids (proline and hydroxyproline) [26]. According to Ref. [27], the amino acid composition of gelatin is similar to that of the parent collagen and consists mainly of repeating sequences of Gly-X-Y triplets, where X is mostly proline and Y is mostly hydroxyproline. Amino acid analysis of chicken skin gelatin also revealed a high content of glycine (33.70%) and hydroxyproline (12.13%), and the high content of imino acids and alanine may contribute to the high viscoelastic properties of gelatin [6]. Giménez et al. [43] also reported that alanine plays an important role in the viscoelasticity of gelatin.

High amino acid content is associated with high gel strength [42]. In particular, a high content of hydroxyproline affects the viscoelastic properties of the gelatin and its ability to develop a strong gel structure [44]. This is because imino acids are important for the renaturation of gelatin subunits during gelling [40]. Ref. [45] reported that hydroxyproline is the major determinant of stability due to its ability to generate hydrogen bonds through its hydroxyl group, but proline plays an important role as well.

During gelling, the broken chains in gelatin undergo a conformational disorder-order transition and partly regenerate the collagen triple-helix structure [46]. The gelling process forms the thermoreversible networks by associating helices in junction zones that are stabilized by hydrogen bonds [47]. The super-helix structure of gelatin gels is important, as it is also stabilized by steric restrictions. Pyrrolidine rings of the hydroxyproline and the hydrogen bonds formed between the amino acid residues are responsible for imposing the restrictions [4]. Ref. [48] stated that hydroxyproline confers greater stability than proline in the Y position in the stereochemical restrictions of the imino acid rings. This is because the hydroxyl groups of hydroxyproline residues point outward from the triple helix, which means that there are no direct hydrogen bonds with any other group within the molecule. This scenario led to the proposal that its effect is mediated through the bridging of water molecules. The triple helix is stabilized by the hydrogen bonding that occurs every three residues, mainly between the NH backbone of glycine and the CO backbone of the residue in the X position of the adjacent chain (Gly)NH----C=O(X). Water molecules form hydrogen bonds between the hydroxyl groups of hydroxyproline and the peptide backbone CO and NH groups within each chain and between different chains, or they bridge the back-bone carbonyl group of the adjacent proline residue [49]. Bella et al. [49] noted that the water bridge refers to the association of hydrogen bonded water molecules that link two different groups that are capable of hydrogen bonding in the triple helix.

The L* and b* values of ALDS were significantly higher than those of ADS and BG (Table 4). ALDS also had a significantly lower a* value than that of ADS and BG. Gelatin from tiger toothed croaker skin (75.41), pink perch skin (71.74) [24], shark skin (82.8), rohu (78.1), and tuna (75.3) [50] had higher lightness values (L*) than ADS, ALDS, and BG. Gelatin from tiger toothed croaker skin (2.79), pink perch skin (2.74) [24], shark skin (1.65), and tuna skin (9.13) had high redness values (a*), whereas that of rohu skin (–0.75) [51] was greener and similar to the values for ADS, ALDS, and BG.

Gelatin from tiger toothed croaker skin (19.25), pink perch skin (22.07) [24], shark skin (18.3), tuna skin (5.36), and rohu skin (5.36) [50] had higher yellowness values (b*) compared to the bluer colour of ADS, ALDS, and BG.

Table 4. Colour measurement of ADS (Acid), ALDS (Alkaline) and BG (Bovine)

Treatments	L*	a*	b*
ADS	29.29 ± 0.89^{a}	-1.24 ± 0.08^{a}	-2.57 ± 0.07^{a}
ALDS	36.58 ± 0.04^{b}	-2.23 ± 0.07^{b}	3.12 ± 0.11^{b}
BG	16.89 ± 1.05^{c}	-0.90 ± 0.07^{c}	-1.09 ± 0.10^{c}

[abc]Values are means of three replicates ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

The colour and clarity of gelatin gels are important aesthetic properties. According to Ref. [51], the colour of gelatin depends on the raw materials and method of extraction used. The coloration of the gelatin increases with increasing time due to the Maillard reaction, which occurs between protein and traces of carbohydrate that are found in the raw material [41]. The gelatin colour will not affect the functional properties of gelatin, but light coloured gelatin is preferable for easy incorporation of gelatin into food systems without imparting a strong colour attribute to the product [50]. Ref. [52] reported that higher temperature is more likely to induce non-enzymatic browning reactions than lower temperature. A higher extraction temperature results in more free amino groups due to the hydrolysis process, and these amino groups undergo a browning reaction with the carbonyl compounds in skin. This results in yellowness (b*) and redness (a*) values in gelatin that are extracted at higher temperatures [28].

According to Ref. [27], the turbidity and dark colour of gelatin is commonly caused by inorganic, protein, and mucosubstance contaminants that are introduced and/or not removed during the extraction process. The efficiency of the filtration process during gelatin extraction also affects the degree of clarity of the gelatin solution [33]. During gelatin extraction, filter paper (Whatman no. 4) is used for filtration. According to Ref. [53], pigments can be removed during pretreatment prior to gelatin extraction.

The pH of ADS and BG was significantly lower than that of ALDS (Table 5). Ref.. [50] reported pH values of shark skin, rohu skin, and tuna skin gelatin to be within 4.17 to 4.34, which indicates that they are Type B gelatin. pH of black tilapia skin (3.91) and red tilapia skin (3.05) gelatins treated with a mixed pretreatment differed from pH of tilapia skin gelatin (3.77) [20]. Differences in pH of gelatin can due to the type and strength of acids employed during the extraction procedures. Ref. [54] suggested that use of two pretreatments provides a proper pH for extraction, as during the extraction process some cross-linkages could be further destroyed but with minimum breakage of polypeptide chains. The pH of ALDS (8.34) was within the range of pH 8 to 9, which shows that the alkaline and acid pretreatment was not sufficient to achieve a near neutral pH. A higher pH of gelatin can be due to inefficiency of washing after the chemical treatments during the preparation of raw materials or before the extraction [55]. Alkaline followed by acid pretreatment of pollock skin resulted in a high yield of gelatin as well as a high bloom value [56].

Table 5. pH and viscosity value of ADS (Acid), ALDS (Alkaline), and BG (Bovine) gelatin gels

Treatments	pH	Viscosity (mPAs)
ADS	5.31 ± 0.04^{a}	13.51 ± 0.56^{a}
ALDS	8.34 ± 0.23^{b}	12.35 ± 0.35^{a}
BG	4.90 ± 0.05^{a}	3.62 ± 0.02^{b}

[abc]Values are means of three replicates ± standard deviation.
Different letters in the same column indicate significant differences ($p < 0.05$).

Ref. [57] found that at extreme pH values, the amount of basic or acidic amino acid residues available for bond formation decreased rapidly, resulting in a sharp decrease in the firmness of the gelatin gel. Choi and Regenstein [58] reported a marked decrease in gel strength of gelatin gels below pH 4. According to Pang et al. [59], the microstructure of 1% gelatin gels had a more three-dimensional network at pH 5.3, and at pH 4.6 the gelatin gels appeared to form more strands than at pH 3. The microstructure of 1% gelatin gels at pH 8.0 was dense; some large pores were present, but individual strands still could be observed. Ref. [60] reported that microstructure can be related to gel strength, as a gelatin with denser strands has higher gel strength than a gelatin with looser strands. Shakila et al. [61] found that bloom value increased with pH until reaching a maximum at pH 7.0, at which point it decreased at pH values higher than 7.0. This observation could explain why ALDS with pH 8.0 had a much lower bloom value than ADS with pH 5.31. A more compact and stiffer gel can be formed by adjusting the pH close to its isoelectric point, where proteins are more neutral and gelatin polymers are closer to each other [19].

The viscosity of ADS was significantly higher ($p < 0.05$) than that of ALDS and BG (Table 5). In addition to gel strength or bloom value of gelatin, viscosity is another important commercial property [13]. Ref. [50] reported that viscosity of gelatin from shark skin, rohu skin, and tuna skin gelatin was < 13.0 cP. Most commercial gelatin viscosities are up to 13.0 cP [14]. The viscosities of most commercial gelatins are partially controlled by molecular weight and molecular size distribution [62]. The viscosity of red tilapia skin gelatin (3.20) is categorized as low, and that of black tilapia skin gelatin (7.12) is mid-range [20]. Viscosities of gelatin from lizardfish scales (7.50) [60], grouper bones (18.5), red snapper bones (15.3) [61], tiger-toothed croaker skin (10.53), and pink perch skin (8.47) [24] are all lower than the values for ADS and ALDS.

Ref. [33] reported that gelatins with a higher proportion of large molecules such as β-chains have higher viscosities. Low viscosity gelatins usually result in short brittle gels, whereas high viscosity gelatins produce tougher and extensible gels [63]. Jamilah and Harvinder [20] found that the effect of pH on viscosity was minimum at the isoionic point of gelatin and maximum at pH 3 and pH 10.5. Ref. [39] reported that minimum viscosity was observed in the pH range of 6 to 8. The variation of viscosity is due to the difference in pH of the extracted gelatin rather than to species or parts of the raw material [20].

4 Conclusion

The results of this study show that duck skin is suitable as a raw material for gelatin production. In particular, ADS had a significantly higher bloom value compared to ALDS (364.10 g and 205.13 g). This is also corresponded to higher hydroxyproline content of ADS compared to ALDS (13.84 g/100g and 10.25 g/100g). There is no significant difference between viscosity of ALDS and ADS. Both ALDS and ADS also had significantly higher viscosities than BG. These results show that duck skin is a potential raw material for gelatin production

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Conflict of Interest
The authors declared that present study was performed in absence of any conflict of interest.

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Author Contribution
NYT performed the experiments and wrote the draft of manuscript. NH designed and supervised the experiments and reviewed the manuscript. AHMS, AMN and ZZ reviewed and update the manuscript.