



Extraction and characterization of gelatin from black-bone chicken by-products

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Abstract In this study, gelatins from black-bone chicken feet and skin (BCFG and BCSG) were extracted using different NaOH concentrations, and their physicochemical properties were characterized and compared to commercial bovine gelatin (BG). It was found that the yield of BCFG was higher than BCSG, however, it contained higher amount of ash. All studied gelatins were composed of two distinct α -chains, while β -chain and γ -chain were not present. The BCFG and BCSG were found to have lower pH, lower hydroxyproline content and lower thermal stability, but higher gel strength as compared with the BG. The colors of BCFG and BCSG were slightly darker than BG. The NaOH concentration did not show strong influence on physicochemical properties of the extracted gelatins, however, thermal stability and gel strength of BCSG tended to decrease with increasing of NaOH concentration. These findings suggested that black-bone chicken feet and skin could be a great source for the production of gelatin.

Keywords Black-bone chicken · Collagen · Gelatin · Gel strength · Amino acid · Thermal stability

Introduction

Black-bone chicken, a breed of chicken with black skin, meat and bone, is well known in Asia as healthy food and traditional medicine. Recently, many studies demonstrated that it composes of active peptides and amino acids that can be used for health promotion. Tian et al. (2007) reported that black-bone chicken contains higher amount of carnosine; a natural dipeptide playing physiological role such as physiological buffer in muscle, anti-oxidant, anti-glycosylation and neurotransmitter function, than normal chicken and it would be better breed for carnosine supplement. Tu et al. (2009) isolated melanin from the muscles of black-bone chicken and found that the isolated pigment contains protein and many metal ions, and it exhibits strong antioxidant activity. Gu et al. (2012) isolated low molecular weight peptides from black-bone chicken and tested for antioxidant and angiotensin I-converting enzyme (ACE) inhibitory activities. The results obtained in this study indicated that black-bone chicken muscle is a good protein source for active peptides by enzyme hydrolysis. Liu et al. (2013) reported that black-bone chicken peptides exhibited a strong antioxidant activity. Results from this study revealed that it is feasible to produce natural antioxidants from black-bone chicken muscle by enzymatic hydrolysis. Besides peptides and amino acid, Tian et al. (2011) reported that lipid from black-bone chicken contains higher essential fatty acids than those of common chicken, and arachidonic acid was the dominant fatty acid. These fatty acids were found to have anti-fatigue and anti-hypoxic activities, and inhibit isoproterenol-induced oxygen consumption.

Driven by health concern and evidence of biological activities of black-bone chicken previously reported in literatures, it has been receiving an attention and increasing

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in demand, resulting in the expansion of breeding and production. High production of black-bone chicken led to delivering of abundant by-products such as head, skin, bone and feet. These by-products were mostly underutilized causing high cost of waste management. Even some of them were utilized, only to low value product such as animal feed was produced. Since black-bone chicken consists of unique active peptides, instead of disposal as waste, extraction of gelatin could be an effective choice to utilize and add the value of these by-products.

Gelatin is a soluble protein obtained through partial hydrolysis of collagen; the fibrous protein constituent in bones, connective tissue, cartilages and skins of animal. Due to its functional properties such as gelling, foaming, stabilizing and encapsulating agents, gelatin has been widely applied in many fields including food, pharmaceutical and cosmetic industries. Furthermore, since many studies have been reported bioactivities of gelatin hydrolysate; the product from enzymatic hydrolysis of collagen or gelatin, such as antioxidant, antimicrobial and antihypertensive activities (Cheng et al., 2009; Gómez-Guillén et al., 2011; Saiga et al., 2008). Gelatin and gelatin hydrolysate, hence, have been widely used as ingredient in functional foods recently. Commercially, gelatin is produced from skin and bone of bovine and porcine. However, due to the effects of bovine spongiform encephalopathy, foot-and-mouth disease and religious reasons, attention nowadays is being focused on alternative sources such as fish, poultry, alligator, giant red sea cucumber and octopus (Gómez-Guillén et al., 2011; Jridi et al., 2015). The properties of these gelatins are varied due to amino acids and peptides compositions resulting from production processes and intrinsic factors such as species and age of animals. The property of gelatin from different sources, hence, should be characterized in order to apply appropriately.

To date, studies of gelatin and collagen from chicken by-products have been reported (Rafieian et al., 2015; Widyasari and Rawdkuen, 2014; Zhou et al., 2016). However, there is no study of gelatin from black-bone chicken. As the previous studies revealed that amino acid and peptides of black-bone chicken were different from normal chicken and could have potential to produce gelatin with good functional properties. Furthermore, it has been reported that functional properties and biological activity (a consequence of amino acid and antioxidative peptides composition) of gelatin and gelatin hydrolysates are strongly influenced by pretreatment method in extraction process (Abedinia et al., 2017; Erge and Zorba, 2018; Hao et al., 2009). Therefore, the objective of this study was to extract gelatin from black-bone chicken by-products, i.e., feet and skin (BCFG and BCSG), using various alkali pretreatments and compare their properties with

commercial bovine gelatin (BG). The expectation outcome of this study is to reduce by-products and investigate possibility of finding new source of gelatin with specific properties for applications in industries.

Materials and methods

Materials

Fresh skins and feet of black-bone chicken were obtained from chicken slaughterhouse. They were frozen immediately upon arriving at laboratory and kept below $-18\text{ }^{\circ}\text{C}$ before further experiment.

Sample preparation

The frozen skins and feet were thawed at $4\text{ }^{\circ}\text{C}$ and cleaned by running water before chopping into small pieces and then freeze drying. Dried skins and feet were ground and defatted by Soxhlet method (AOAC, 1990), then kept in hermetically sealed aluminium foil bag prior to gelatin extraction.

Gelatin extraction

Gelatin extraction was carried out according to the method proposed by Sarbon et al. (2013) with slight modification. Firstly, defatted skins or feet (14 g) were pretreated by mixing with 200 ml of different concentrations of sodium hydroxide (0.025, 0.050, 0.075 N) and slow stirring at $22\text{ }^{\circ}\text{C}$ for 80 min. Consequently, the mixtures were centrifuged at $3500\times g$ for 10 min, rinsed with 200 ml distilled water, and again centrifuged at $3500\times g$ for 10 min.

The pretreated samples were mixed with 200 ml of 0.15% (v/v) sulfuric acid solution, slow stirring for 40 min and $3500\times g$ centrifuging for 10 min. Then, the obtained pellets were mixed with 200 ml of 0.7% (w/v) citric acid solution for 40 min and performed centrifugation at $3500\times g$ for 10 min. This step was repeated three times. After that, the pellets were rinsed with 200 ml distilled water before centrifugation at $3500\times g$ for 10 min.

At the final step of extraction, the samples were mixed with 200 ml of $45\text{ }^{\circ}\text{C}$ distilled water for 15 h without stirring. Then the mixtures were cooled at room temperature before mixing with 4 g of activated carbon for 20 min and filtered through Whatman No.4 paper. The mixtures were concentrated by vacuum evaporation at $45\text{ }^{\circ}\text{C}$ using rotary evaporator (Buchi Rotavapor R200/R205, Flawil, Switzerland) to obtain approximately 20 ml of concentrated mixtures. The concentrated mixtures were dried by freeze drying (ALPHA 1-2 LD plus, CHRIST, Germany)

and ground to obtain black-bone chicken feet gelatin (BCFG) or black-bone chicken skin gelatin (BCSG).

The yield of gelatins (% , dry basis) was calculated by following equation:

$$\text{Yield} = \frac{W_g}{W_d} \times 100 \quad (1)$$

where w_g is weight of gelatin (g) and w_d is weight of dried skins or feet (g).

Gelatin characterization

Proximate analysis

Proximate analysis, i.e., moisture, protein, fat and ash contents, was carried out by Association of Official Analytical Chemist (AOAC) methods (AOAC, 1990).

Amino acid analysis

Amino acid content of gelatins was determined using HPLC (Waters: Alliance, Waters, UK, Hertfordshire, UK) with an integrated detector (Dual I absorbance-Waters 2487) and separation module (Waters 2695). Gelatin samples were hydrolyzed to get free amino acid by mixing with hydrochloric acid, tightly capped and heated in an oven at 110 °C over 18 h. Nineteen amino acids were analyzed by the method of Herbert et al. (2000).

Protein pattern

Protein pattern was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method by Laemmli (1970). Samples of 1 g were homogenized using an IKA homogenizer (IKA Works Asia, Bhd, Malaysia) in 19 ml of a 5% SDS solution for 1 min and then heated at 95 °C for 10 min. The samples were then centrifuged at 10,000×g for 10 min. Samples (15 µg) were loaded onto 4% stacking and 7.5% running acrylamide gels. The gels were run at a constant voltage of 100 V, stained with 0.125% Coomassie brilliant blue R-250, and destained with a solution containing distilled water, ethanol and acetic acid at a ratio of 6.5:2.5:1.

pH determination

Gelatin solution was prepared by dissolving 1 g of gelatin in 100 ml distilled water at 60 °C and then cooled down at room temperature. The pH of solution was measured using pH meter (CyberScan pH1000, Metrohm, Switzerland).

Thermal analysis

Thermal property of gelatin was measured using differential scanning calorimeter (DSC) (Mettler Toledo STARE System DSC 1 Module, Mettler Toledo, Switzerland). Before the measurement, the DSC was calibrated by indium and zinc. Gelatin sample of 3.0 mg was weighed in 20 µl aluminium pan and hermetically sealed. The sample pan was then subjected to DSC and scanned from 20 to 95 °C with heating rate of 1 °C/min. The denaturation of gelatin was observed by the occurrence of endothermic peak. The helix–coil transition temperature (T_m) was estimated by peak temperature and the enthalpy change (ΔH) was calculated by integrating peak area.

Gel strength determination

The gelatin sample of 7.5 g was dissolved in 105 ml distilled water to get final concentration of 6.67% (Sarbon et al., 2013). The sample was kept at room temperature for 3 h and then heated at 60 °C for 20 min to allow complete dissolution. The solution was cooled down at room temperature and subjected to refrigerator at 10 °C for 16 h before gel strength measurement. The gel strength was measured by Texture analyzer (TA-XT plus Texture Analyzer, Stable Micro Systems, UK) using radius cylinder probe (P/0.5R) at speed of 0.5 mm/s to 4 mm depth of sample.

Color measurement

The gelatin gel was prepared according to the sample for gel strength measurement. The color of sample was measured using spectrophotometer (Miniscan EZ 4500L spectrophotometer, HunterLab, Virginia, USA). The sample color was reported in CIE Lab system (L^* , a^* , b^*), where L^* represents lightness and parameters a^* and b^* are redness/greenness and yellowness/blueness, respectively. The color of gelatin was also expressed in terms of hue angle (h°) and total color difference (ΔE^*).

The hue angle was calculated by Eq. (2). It is represented in degrees and corresponding to the three-dimensional diagram of colors, where 0° is the red, 90° is the yellow, 180° is the green and 270° is the blue colors (Silva et al., 2014).

$$h^\circ = \tan^{-1} \left(\frac{b^*}{a^*} \right) \quad (2)$$

The ΔE^* was calculated as follows:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

where ΔL^* , Δa^* and Δb^* are the differences between the color parameter of the sample and that of the white standard ($L^* = 92.48$, $a^* = -1.10$, $b^* = 0.30$).

Statistical analysis

All experiments were done triplicate and analysis of variance (ANOVA) using complete randomized design was conducted and the differences between treatments were compared by Duncan's multiple range test at 95% confident interval ($p < 0.05$) (STATISTICA 10.0 Trial Version, StatSoft, Inc., Tulsa, OK, USA).

Results and discussions

Yield

The yields of BCFG and BCSG were ranged from 9.53 to 10.59% and 6.60 to 7.23% (dry basis), respectively (Table 1). It showed that the yield of gelatin was dependent on raw material, and black-bone chicken feet delivered higher yield in comparing to skin. For the gelatin from chicken feet, the study by Almeida and Lannes (2013) reported that the yields of gelatin were also different among different parts of chicken feet.

Besides raw material, pretreatment process is another factor that affected on gelatin yield. In gelatin production process, raw materials can be pretreated with either acid, alkaline or both to allow the swelling of collagen before thermal hydrolysis of collagen to gelatin. In this study, chicken feet and skin were pretreated with different concentrations of NaOH and its effect on yield was investigated. The result showed that there was no significant difference of gelatin yield among the gelatins pretreated with different NaOH concentrations (Table 1). However, it was observed that the yield of gelatins tended to increase with the increasing of NaOH concentration. This suggested

that higher concentration of NaOH could enhance weakening and solubility of collagen structure, which led to an increase of collagen hydrolysis. Similarly, previous studies used NaOH solution to eliminate non-collagen materials in order to provide optimal condition of collagen and gelatin extractions. It was found that the extraction yield increased with increasing of NaOH concentration, however, very high concentration of NaOH could cause inverse effect (Erge and Zorba, 2018; Shon et al., 2011).

In addition to pretreatment process, the yield of chicken gelatin has been reported to be influenced by extraction method. Sarbon et al. (2013) reported that the yield of gelatin from chicken skin was 16.00% (dry basis) for the extraction by hot water, while work from Kim et al. (2012) showed that the yield of gelatin from chicken skin was ranged from 1.74 to 4.43% (wet basis) for the extraction by different combinations of pH and temperature conditions. Widyasari and Rawdkuen (2014) extracted gelatin from chicken feet and found that the yield of 12.64% (dry basis) was obtained by acid extraction, while the lower yield of 12.37% (dry basis) was obtained by ultrasound assisted extraction.

Proximate analysis

Proximate compositions of the studied gelatins are given in Table 1. In this study, fat was not found in all gelatins. Protein and ash contents are the important components relating to the purity that affect the properties of gelatin. As observed in BG, BCSG had high protein content (89.33–92.26%) and low ash content (0.93–1.20%). This result coincided with the compositions of gelatins from normal chicken wastes that have been reported to contain protein ranging from 80 to 90% and ash content lower than 5% (Rafieian et al., 2015; Sarbon et al., 2013; Widyasari and Rawdkuen, 2014). On the other hand, low protein content (75.13–76.37%) and high ash content (14.55–16.34%) were found in BCFG. Since whole feet

Table 1 Yield and proximate composition of gelatins

Gelatin	NaOH concentration (N)	Yield (% , dry basis)	Composition (%)		
			Moisture	Protein	Ash
BG			9.06 ± 0.21 ^a	89.84 ± 0.31 ^a	0.95 ± 0.02 ^c
BCFG	0.025	9.53 ± 2.33 ^a	8.78 ± 0.34 ^a	76.37 ± 0.00 ^b	14.95 ± 0.09 ^b
	0.050	9.96 ± 2.58 ^a	10.04 ± 1.17 ^a	75.13 ± 0.05 ^b	14.20 ± 0.02 ^c
	0.075	10.59 ± 1.65 ^a	8.58 ± 1.04 ^a	75.82 ± 0.25 ^b	15.90 ± 0.11 ^a
BCSG	0.025	6.60 ± 0.87 ^b	9.33 ± 0.18 ^a	89.52 ± 0.13 ^a	1.06 ± 0.01 ^{de}
	0.050	7.11 ± 0.87 ^b	9.20 ± 0.77 ^a	89.33 ± 0.56 ^a	0.93 ± 0.13 ^c
	0.075	7.23 ± 1.15 ^b	7.37 ± 0.07 ^a	92.26 ± 4.54 ^a	1.20 ± 0.06 ^d

Values are mean ± standard deviation (n = 3)

^{a,b,c,d,e}Different letters within the same column indicate significant difference ($p < 0.05$)

were used in the extraction of BCFG, hence, high amount of ash could be due to the mineral in bone. This result agreed with the study by Almeida and Lannes (2013) who compared compositions of gelatin from different parts of chicken feet, i.e., whole feet, metatarsus, paws, and skin and tendons. In their study, the lowest ash content was found in skin and tendons, whereas the highest ash content was found in metatarsus due to large amount of mineral in bone. Regarding to the gelatin yield result, although the yield of BCFG was higher than that of BCSG, however, the level of ash in BCFG was found to be higher than recommended limit of ash in edible gelatin (not exceed 2%) (GMIA, 2012). The extraction method for BCFG is needed to be improved, alternatively, chicken feet bone should be removed prior the extraction.

It has been reported that NaOH concentration strongly influenced on gelatin composition, the protein content significantly increased with increasing of NaOH concentration (Erge and Zorba, 2018). However, among the BCFG and BCSG pretreated with different NaOH concentrations in this study, there was no significant difference of protein content, and small deviation of ash content (Table 1). This suggested that although increasing of NaOH concentration could enhance removing of impurities and weakening of collagen structure, however it did not influence the leaching of non-collagen protein or mineral from raw materials, as a consequence, the gelatin composition was not affected. The different result could be due to the different ranges of NaOH concentrations used in this study and previous study.

Amino acid analysis

In this study, nineteen amino acids and imino acid (proline + hydroxyproline) were quantified (Table 2). All studied gelatins contained high amount of arginine, glycine and hydroxyproline but low amount of cystine, methionine and tryptophan. Although, some differences in amino acid composition among the studied gelatins were noticeable, however, there was no clear influence of NaOH pretreatment or tendency of amino acid composition in gelatin pretreated with different NaOH concentrations. Similar result was reported by Abedinia et al. (2017), Hao et al. (2009) and Lee et al. (2012). Hao et al. (2009) found that the effect of pretreatment methods on amino composition was not significant, alternatively, it strongly affected on molecular weight distribution of gelatin.

Imino acid is a component that plays an important role in thermal stability of gelatin. It showed that imino acid content in BCFG was significantly higher than in BG. This result coincided with the study by Rafeian et al. (2013) who found that imino acid content in chicken gelatin was higher than that of bovine gelatin. However, the

hydroxyproline content in BG was found to be higher than that of the two black-bone chicken gelatins.

Protein pattern

Gelatin is made of amino acids fractions to form polypeptide, varying in molecular weight from 15 to 300 kDa. In SDS-PAGE, the polypeptide chain is classified by ranges of molecular weight as γ -, β -, α -chains and lower molecular weight fractions. The α -chain is a monomer of polypeptide having an approximate molecular weight of 100 kDa, while the β -chain and γ -chain (dimers and trimers of α -chains) have a molecular weight of approximately 200 and 300 kDa, respectively. It was found that the BCSG, BCFG and BG contained two distinct α -chains with molecular weight ranged from 110 to 130 kDa, while protein bands with molecular weight higher than 200 kDa which corresponded to β -chain and γ -chain were not observed (Fig. 1). Furthermore, α -chains band of the BCFG and BCSG was found to have slightly higher molecular weight than that of the BG (Fig. 1). Different observation was reported by Zhou et al. (2016), who found that collagens from chicken feet were mainly composed of at least two different α -chains and also β -chain and γ -chain. Widyasari and Rawdkuen (2014) reported that gelatin from chicken feet was composed of α -chain and β -chain. In addition, Yang et al. (2017) revealed that duck feet gelatin contained two distinct α -chains, β -chain and γ -chain. The main factor affecting protein pattern of gelatin was the hydrolysis during the extraction process that contributed to the splitting of peptide chains. The absence of β -chain and γ -chain in the BCFG and BCSG in this study suggested that most of inter-chain crossed link was damaged during extraction process.

Among the BCFG and BCSG pretreated with different concentrations of NaOH, the α -chain band intensity of the gelatins slightly increased with the increasing of NaOH concentration from 0.025 to 0.050 N but decreased with further increasing of NaOH concentration from 0.050 to 0.075 N (Fig. 1). The increasing of α -chain band intensity with the increasing of NaOH concentration might be due to higher cleavage of inter-chain crossed link during thermal hydrolysis, resulting from greater swelling of collagen structure at higher NaOH concentration. However, further increasing of NaOH concentration could cause degradation of α -chains to lower molecular weight fractions as observed by lower intensity of α -chain band (Zhou and Regenstein, 2005).

Since gelatin is produced by partial hydrolysis of collagen, hence, the structure of gelatin is dependent on collagen source. Type I collagen is a major structural component of skin, bone and connective tissues, generally consisting of two α 1-chains and one α 2-chain which can be

Table 2 Amino acid composition of gelatins

Amino acid	Residues/1000 residues						
	BG	BCFG NaOH concentration (N)			BCSG NaOH concentration (N)		
		0.025	0.050	0.075	0.025	0.050	0.075
Alanine	127.20 ^b	87.15 ^c	61.94 ^e	74.80 ^d	62.93 ^e	142.84 ^a	142.79 ^a
Arginine	120.95 ^d	143.42 ^b	163.20 ^a	145.45 ^b	165.07 ^a	128.43 ^c	128.84 ^c
Aspartic acid	47.58 ^a	39.28 ^b	34.05 ^c	31.51 ^c	49.73 ^a	48.82 ^a	49.20 ^a
Cystine	7.49 ^{de}	9.15 ^c	6.39 ^e	13.52 ^a	11.17 ^b	7.70 ^d	7.93 ^d
Glutamic acid	84.26 ^c	84.42 ^c	86.03 ^{bc}	83.46 ^c	91.34 ^a	89.66 ^{ab}	89.68 ^{ab}
Glycine	175.74 ^b	173.62 ^b	184.41 ^a	165.31 ^c	186.56 ^a	186.92 ^a	186.47 ^a
Histidine	26.12 ^d	32.29 ^a	28.26 ^c	31.68 ^b	30.78 ^b	29.22 ^{bc}	29.80 ^{bc}
Isoleucine	17.56 ^a	14.10 ^d	9.65 ^e	16.10 ^{bc}	15.44 ^c	16.07 ^{bc}	16.77 ^b
Leucine	26.23 ^{ns}	26.14 ^{ns}	26.17 ^{ns}	26.90 ^{ns}	27.60 ^{ns}	26.53 ^{ns}	26.60 ^{ns}
Lysine	34.97 ^c	29.17 ^d	36.05 ^{bc}	44.76 ^a	33.89 ^c	36.89 ^b	36.34 ^b
Methionine	6.56 ^c	10.67 ^a	9.06 ^b	11.11 ^a	11.33 ^a	9.97 ^{ab}	9.99 ^{ab}
Phenylalanine	16.30 ^d	21.80 ^b	21.34 ^b	26.51 ^a	22.78 ^b	18.35 ^c	18.81 ^c
Proline	52.78 ^e	112.41 ^a	114.89 ^a	95.83 ^b	70.90 ^c	57.35 ^d	52.44 ^e
Serine	27.40 ^a	23.66 ^c	23.40 ^c	23.73 ^c	25.61 ^b	24.37 ^{bc}	24.51 ^{bc}
Threonine	48.06 ^a	29.09 ^{de}	34.38 ^b	30.24 ^{cd}	32.16 ^c	27.04 ^e	30.23 ^{cd}
Tryptophan	0.55 ^{ns}	0.67 ^{ns}	0.91 ^{ns}	0.83 ^{ns}	0.63 ^{ns}	0.70 ^{ns}	0.75 ^{ns}
Tyrosine	39.78 ^c	38.84 ^c	32.40 ^d	60.72 ^a	46.66 ^b	28.89 ^e	25.76 ^f
Valine	20.43 ^a	19.23 ^{ab}	16.25 ^c	19.36 ^{ab}	18.53 ^{abc}	17.81 ^{bc}	17.76 ^{bc}
Hydroxyproline	120.05 ^a	104.91 ^c	111.26 ^b	98.28 ^d	96.87 ^d	102.47 ^c	105.78 ^c
Imino acid	172.83 ^d	217.32 ^b	226.15 ^a	194.11 ^c	167.77 ^d	159.83 ^e	158.21 ^e

Values are means of triplicates

^{a,b,c,d,e}Different letters within the same row indicate significant difference ($p < 0.05$)

^{ns}indicates non-significant difference ($p > 0.05$)

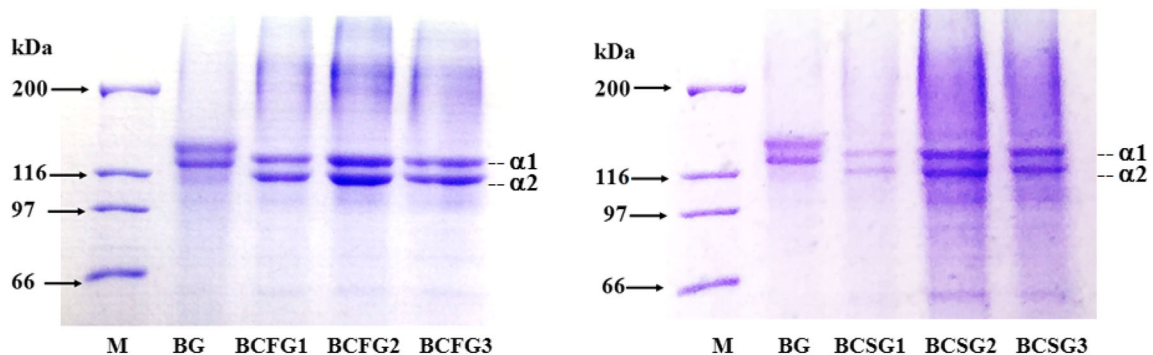


Fig. 1 SDS-PAGE pattern of gelatins; protein standard marker (M), commercial bovine gelatin (BG), black-bone chicken feet gelatins pretreated with 0.025–0.075 N NaOH (BCFG1–3) and black-bone chicken skin gelatin pretreated with 0.025–0.075 N NaOH (BCSG1–3)

observed by two-fold intensity of $\alpha 1$ -chains greater than of $\alpha 2$ -chain. In this study, the BCSG and BCFG contained two distinct α -chains ($\alpha 1$ and $\alpha 2$), however, two folds intensity of $\alpha 1$ -chains was not observed. Therefore, it was assumed that the collagen from black-bone chicken consisted of not only type I collagen but at least two types of collagen. Similar result has been reported by Cheng et al. (2009) who revealed that normal chicken feet and skin

contained two types of collagen. In addition, it has been reported that type I collagen was a major type collagen from chicken feet and skin (Kim et al., 2012). However, the study by Oechsle et al. (2016) concluded that chicken skin collagen revealed type I and III collagens while chicken bone collagen revealed solely type I collagen.

pH

The ANOVA result suggested that pH value of the BCFG and BCSG was not influenced by NaOH pretreatments ($p > 0.05$), hence only mean values of each type of gelatin were presented (Table 3). The result showed that the pH of BCSG and BCFG were acidic ranging from 3.71 to 4.81, while pH of BG was nearly neutral (6.41). The pH variation is contributed by types and strengths of chemicals (acid or alkaline) used in the extraction process. In addition, washing procedure is important step in removing the acid and/or alkaline residues. The pH of BCFG and BCSG were lower than pH of chicken gelatins previously reported by Kim et al. (2012) and Widayari and Rawdkuen (2014). In those studies, the pH of chicken gelatins to be close to neutral, ranging from 6.1 to 6.8. The low pH value of the BCSG and BCFG in this study could be a result of subsequent acid treatment after alkali pretreatment in the extraction process.

Color

The preference of gelatin color depended on application. In general, gelatin with light and clear color is preferred since it can be incorporated in foods with no influence on product color. In this study, color of gelatins was expressed in term of lightness (L^*), redness (a^*), yellowness (b^*), total color difference (ΔE^*) and hue angle (h°). It was found that there was no significant difference of color of gelatins pretreated with different NaOH concentrations (data not shown) ($p > 0.05$). This result agreed with the study by Hao et al. (2009) who studied the effect of two alkalis pretreatment (NaOH and $\text{Ca}(\text{OH})_2$) and concluded that color of gelatin was not influenced by alkali concentration but types of alkali. However, Erge and Zorba (2018) reported that there was interaction effect of NaOH concentration and extraction temperature on L^* of gelatin.

Since there was no significant difference of color of gelatins pretreated with different NaOH concentrations in this study, hence, only mean color values of the BCFG and

BCSG were compared with BG (Table 3). The BCFG and BCSG showed slightly lower L^* , but greater b^* and ΔE^* in comparing to BG. The h° value of BG, BCFG and BCSG were 99.29° , 95.43° and 89.71° , respectively. The h° value of 90° represented yellow color, while the value of higher than 90° represented greenish-yellow color, the higher h° , the greater greenish. The result showed that the BG has more greenish than that of BCFG and BCSG. The difference in color of the two extract gelatins from BG could be due to the pigment in raw material that has not been solely removed during the extraction.

Thermal analysis

Thermal stability of gelatin is defined as its resistance to denature, determining by helix–coil transition temperature (T_m), and enthalpy change (ΔH) (Fig. 2).

The T_m of BCFG and BCSG were relatively high (58.47 – 63.17°C) (Table 4) when compared with those of chicken gelatins previously reported (30.99 – 39.83°C) (Kim et al., 2012; Sarbon et al., 2013). Besides intrinsic factors and extraction procedure, concentration of gelatin solution used in the analysis is another factor altering thermal property of gelatin, the higher gelatin concentration, the higher T_m and ΔH (Sarbon et al., 2013). In this study, the gelatin powders were used in the analysis, whereas gelatin solutions with concentration ranged from 30 to 100 g/l were used in the previous studies, therefore, high T_m and ΔH could be observed in this study.

The BCFG and BCSG showed lower T_m and ΔH than the BG (Fig. 2 and Table 4). This indicated that black-bone chicken gelatins had lower thermal stability in comparing to BG. In contrast to the study by Sarbon et al. (2013) who found that gelatin from normal chicken skin had higher T_m and ΔH than bovine gelatin. Imino acid content (proline + hydroxyproline) has been described to play the key roles in thermal stability of gelatin, a gelatin with higher amount of imino acid tends to have higher thermal stability. In this study, the amino acid analysis showed that the BCFG contained higher amount of imino acid (particularly

Table 3 pH and color of gelatins

Gelatin	pH	Color				
		L^*	a^*	b^*	ΔE^*	h°
BG	6.41 ± 0.08^a	52.29 ± 1.18^a	-1.07 ± 0.07^c	6.60 ± 0.55^b	40.47 ± 1.20^b	99.29 ± 0.36^a
BCFG	4.81 ± 0.10^b	47.17 ± 0.97^b	-0.66 ± 0.05^b	7.04 ± 0.62^b	45.81 ± 0.96^a	95.43 ± 0.79^b
BCSG	3.71 ± 0.05^c	47.29 ± 3.12^b	0.04 ± 0.02^a	9.94 ± 0.15^a	46.25 ± 2.60^a	89.71 ± 0.35^c

Values are mean \pm standard deviation

^{a,b,c}Different letters within the same column indicate significant difference ($p < 0.05$)

Fig. 2 DSC thermogram of BCFG and BCSG pretreated with 0.025 N NaOH and BG

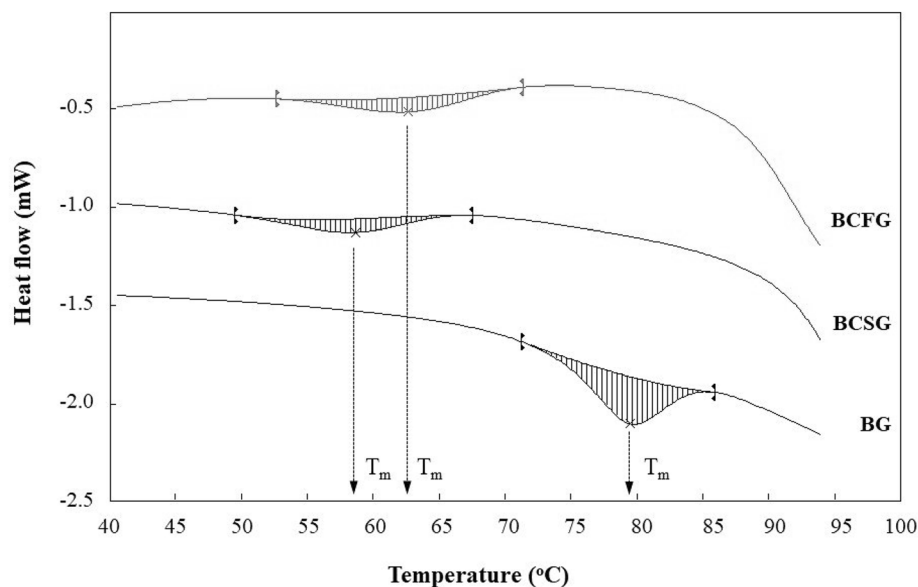


Table 4 Thermal property and gel strength of gelatins

Gelatin	NaOH concentration (N)	Thermal property		Gel strength (g)
		T_m (°C)	ΔH (kJ/kg)	
BG		78.35 ± 0.53^a	1.99 ± 0.12^a	228.8 ± 3.2^c
BCFG	0.025	63.14 ± 0.86^c	0.92 ± 0.04^b	251.0 ± 6.7^a
	0.050	69.77 ± 3.89^b	0.24 ± 0.23^c	254.3 ± 5.9^a
	0.075	67.70 ± 0.59^b	0.43 ± 0.09^c	256.6 ± 4.5^a
BCSG	0.025	58.47 ± 1.33^{de}	1.00 ± 0.03^b	263.5 ± 3.9^a
	0.050	59.42 ± 1.46^d	0.48 ± 0.29^c	249.1 ± 9.9^{ab}
	0.075	55.48 ± 0.87^c	0.37 ± 0.22^c	239.0 ± 6.7^{bc}

Values are mean \pm standard deviation ($n = 3$)

^{a,b,c,d,e}Different letters within the same column indicate significant difference ($p < 0.05$)

proline) than that of BG (Table 2), unfortunately, the greater thermal stability of BCFG was not observed. This could be explained that although proline is important, hydroxyproline is believed to play the utmost important role in stabilization of triple helix due to its hydrogen-bonding ability through $-OH$ group (Cheng et al., 2009; Rafeian et al., 2013). The lower thermal stability of BCFG (including BCSG) than BG might be due to lower hydroxyproline content. Moreover, the slightly lower molecular weight of α -chains of the BCFG and BCSG (Fig. 1) could probably be additional factor that caused lower thermal stability of these two gelatins.

Comparing the two black-bone chicken gelatins, the T_m of BCFG was significantly higher than BCSG (Table 4). This indicated that gelatin from feet had higher thermal stability as compared with gelatin from skin. As mentioned above, the T_m was related to hydroxyproline content and molecular weight distribution, however, the difference in hydroxyproline content and molecular weight distribution

between BCFG and BCSG were not observed. The higher T_m of BCFG could probably be a result of higher ash content in BCFG.

The NaOH concentration did not show strong influence on T_m and ΔH of BCFG and BCSG (Table 4), although ΔH of these gelatins tended to decrease with increasing of NaOH concentration. Erge and Zorba (2018) reported that the effect of NaOH concentration on T_m and ΔH of gelatin was not significant. However, the gelling and melting temperatures of the gelatin were found to decrease with increasing of NaOH concentration due to higher low molecular weight fractions.

Gel strength

Typically, gelatin can be classified by gel strength of gelatin gel at 6.67% (Bloom value) as high bloom (200–300 g), medium bloom (100–200 g) and low bloom (50–100 g). Different gel strengths are used in different

applications. In this study, gel strengths of BCSG and BCFG were ranged from 239.0 to 263.5 g, classifying as high bloom gelatin (Table 4).

Like thermal stability, gel strength of gelatin has been reported to be affected by extraction procedures as well as intrinsic properties of collagen sources. Generally, gelatin with high imino acid content (especially hydroxyproline) and high molecular weight will contribute to high gel strength (Gómez-Guillén et al., 2011; Saiga et al., 2008). Additionally, gelatin with more neutral tended to have higher gel strength (GMIA, 2012; Shon et al., 2011). In comparing to BG, the BCFG and BCSG were found to have more acidic (Table 4), slightly lower molecular weight (Fig. 1) and lower hydroxyproline content (Table 3). Moreover, the T_m and ΔH of BCFG and BCSG were lower than BG. Hence, the lower gel strength of the BCFG and BCSG than BG could be expected. In contrary, the higher gel strength was observed in BCSG and BCFG. Other intrinsic properties could probably be additional factors affecting this property. However, this result agreed with Rafeian et al. (2015) who reported that gel strength of chicken deboner gelatin was higher than commercial gelatin (520 g and 190 g, respectively). Besides, research by Sarbon et al. (2013) showed that gel strength of gelatin from chicken skin was significantly higher than that of bovine gelatin (355 g and 229 g, respectively).

The effect of NaOH concentration on gel strength of BCFG was not significant. However, the gel strength of BCSG tended to decrease with the increasing of NaOH concentration (Table 4). This result coincided with the study by Erge and Zorba (2018) who reported that gel strength of gelatin decreased with increasing of NaOH concentration. The decreasing of gel strength at higher NaOH concentration could be due to a greater degradation of collagen, resulting in α -chains and low molecular weight fractions. In contrast, Shon et al. (2011) reported that gel strength of gelatin increased with small increasing of NaOH concentration, however, after certain amount of NaOH, the gel strength began to decrease. The different ranges of NaOH concentrations and type of raw materials could be additional factors that caused divergent effects of NaOH concentration.

Regarding to the results, it could be conclude that black-bone chicken by-products, i.e., feet and skin, could be an alternative source for production of gelatin with good physicochemical properties. However, slight modification of extraction process of BCFG is required in order to reduce the presence of mineral to the acceptable level.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest in this paper.

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