

Extraction of crude gelatin from duck skin: effects of heating methods on gelatin yield

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ABSTRACT The disposal of by-products of duck production, including duck skin, is a serious concern as it results in environmental pollution. The objectives of this study were to investigate the optimal pretreatment conditions for swelling duck skin and their extraction methods as a novel source. Gelatin was extracted using water bath, sonication, superheated steam, and microwave extraction methods. The gelatin extraction yield and gelatin powder yield were the highest with the superheated steam extraction method. The melting point and gel strength of gelatin extracted using the superheated steam method were the lowest.

The viscosity of gelatin extracted with the superheated steam and microwave extraction methods was higher than that of gelatin extracted with the other methods. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of gelatin extracted using the superheated steam and microwave extraction methods showed more intense bands than those of gelatin extracted using the other methods. Our results showed that gelatin extracted from duck skin using the superheated steam extraction method had optimal physical properties and therefore can be used in meat products.

Key words: duck skin, gel strength, gelatin, microwave, superheated steam

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INTRODUCTION

With increasing global demand for duck meat, the production of duck has steadily increased (Kim et al., 2017; Shin et al., 2019) especially over the last 20 yr (Kim et al., 2016). However, this has negative implications because the disposal of by-products of duck production, including duck skin as biological waste, results in environmental pollution, which is a serious concern in the duck industry (Huda et al., 2013; Shim et al., 2018). Studies have shown that duck skin can be a potential alternative and novel source of collagen and gelatin (Noh et al., 2019). Kim et al. (2017) have reported that the duck skin contains collagen, which can be used as a food additive. Further, Lee et al. (2012) have reported that the duck skin contains an antioxidative peptide that reduces free radical production, lowers blood pressure, and prevents cardiovascular diseases. Therefore, the extraction of gelatin from duck skin is worth studying.

Collagen is a major component of the skin, tendons, and connective tissues, and it accounts for approxi-

mately one-third of the total proteins in the body (Lin and Liu, 2006; Yeo et al., 2014). Generally, collagen is extracted from the above-mentioned parts of animals using suitable heating and acidification treatments. Various functional properties of collagen have been reported, including gelling and film-forming properties, surface behavior, and microencapsulation (Gómez-Guillén et al., 2011; Kim et al., 2016; Mulyani et al., 2017). Studies have evaluated the physicochemical, textural, and sensory properties of gelatin from duck feet and jellies from duck meat (Kim et al., 2014), and collagen from duck feet (Cha et al., 2016). Furthermore, the quality characteristics of low-fat frankfurter prepared using gelatin extracted from duck feet have been analyzed (Yeo et al., 2014). Most of these studies have focused on the extraction and application of collagen from duck feet. Hence, there is a need for studies on the extraction of collagen from duck skin, considering the lack of information in this regard. This alternative application of duck skin will help reduce environmental pollution caused by biological waste of duck production.

The objectives of this study were to characterize and assess the physicochemical properties of crude gelatin extracted using different extraction methods, namely, water bath, sonication, superheated steam, and microwave extraction methods. Our study can provide

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basic information on alternative application of duck skin to obtain gelatin for industrial use.

MATERIAL AND METHODS

Extraction of Gelatin From Pretreated Duck Skin

After washing to remove a curd and visible fat of the duck skin obtained from Farm duck Co. Ltd. (Korea) using tap water, the skin was soaked with various solutions that were adjusted pH 1 to 14 with 0.1 N HCl, 0.1 N NaOH, and distilled water. The process of skin swelling was modified from Park et al. (2013). The skin samples were soaked in 5-fold volume (v/w) of solution of different pH at 18°C for 24 h. The soaked skin samples were then washed using tap water at 18°C (48 h), for neutralization. For gelatin extraction, the skin sample swelled with the pH 1 solution was used, because it resulted in the highest rate of swelling. The skin samples were drained, placed in polyethylene bags, and sealed using a vacuum-packaging system (FJ-500XL; Fujee Tech, Korea). Gelatin was extracted using the following methods: (1) water bath extraction method (JSSB-30T; JS Research Inc., Korea) at 60°C for 10 min, (2) sonication extraction method (CPX5900H-E; Emerson, USA) at 60°C with 40 kHz for 10 min, (3) superheated steam extraction method (DFC-240 W; Naomoto, Japan) at oven temperature of 150°C and steam temperature of 150°C for 10 min, and (4) microwave extraction method (MW25S; LG Electronics Tianjin Appliance Co., Ltd., China) at 2450 MHz and 200 W power for 10 min. The melted duck skin was filtered by medical gauze to remove any contaminants. A filtrate was cooled and coagulated at 4°C for 12 h. After isolated fat upper the coagulated gelatin was removed and collected gel layer, the collected samples were frozen at -70°C and dried at -40°C under 80×10^{-3} Torr using a freeze dryer (VTFD; Ilshin, Korea). For the subsequent analyses, 6.67% crude gelatin samples in distilled water were used.

Rate of Swelling

The rate of swelling of duck skin was determined and calculated with the following formula:

$$\text{Swelling rate (\%)} = \left(\frac{\text{weight of sample after swelling}}{\text{weight of sample before swelling}} \right) \times 100.$$

Extraction Yield

The extraction yield was determined and calculated with the following formula:

$$\text{Extraction yield (\%)} = \left(\frac{\text{weight of sample before drying}}{\text{weight of raw material}} \right) \times 100.$$

Gelatin Powder Yield

The gelatin powder yield was determined and calculated with the following formula:

$$\text{Gelatin powder yield (\%)} = \left(\frac{\text{weight of sample after freeze-drying}}{\text{weight of sample before freeze-drying}} \right) \times 100.$$

pH

Five grams of the pretreated duck skin sample were added to 20 mL of distilled water and homogenized for 60 s (Ultra-Turrax Sk15; Janke & Kunkel, Germany). The pH of the sample was determined using a pH meter (340; Mettler-Toledo GmbH, Switzerland), which was calibrated using buffer solutions of pH 4, 7, and 10.

Melting Point

After measuring the temperature at start and end of melting, the average temperature of the melting point was used. The temperature of melting point was observed using a melting point analyzer (ATM-01, AS ONE, Japan).

Gel Strength

Cubes (2.0 cm × 2.0 cm × 2.0 cm) of the 6.67% crude gelatin gel samples were used to assess the gel strength at 18°C using a texture analyzer (TA-XT2i; Stable Micro Systems Ltd., England). The shear force (kg) of samples was calculated using the maximum force required to shear through each sample. To determine this, a 10-mm depression was created at the center the gelatin cubes at a rate of 0.5 mm/s using a probe of 10-mm diameter.

Apparent Viscosity

The apparent viscosity of the gelatin samples was measured using a rheometer (DV3THB; Brookfield Engineering Laboratories, Middleborough, MA, USA) at 35°C for 10 s. The apparent viscosity was assessed at a constant shear rate of 50 s⁻¹ for 30 s. The maximum apparent viscosity is presented as mPa · s.

Color Values

The color values (i.e., the CIE L*, a*, and b* values) of the gelatin samples were measured using a colorimeter (Minolta Chroma meter CR-400; Minolta Ltd., Japan; illuminate C was calibrated using a white plate; L* = +97.83, a* = -0.43, and b* = +1.98). The CIE L*, a*, and b* values represent the mean intensity of lightness, redness, and yellowness, respectively.

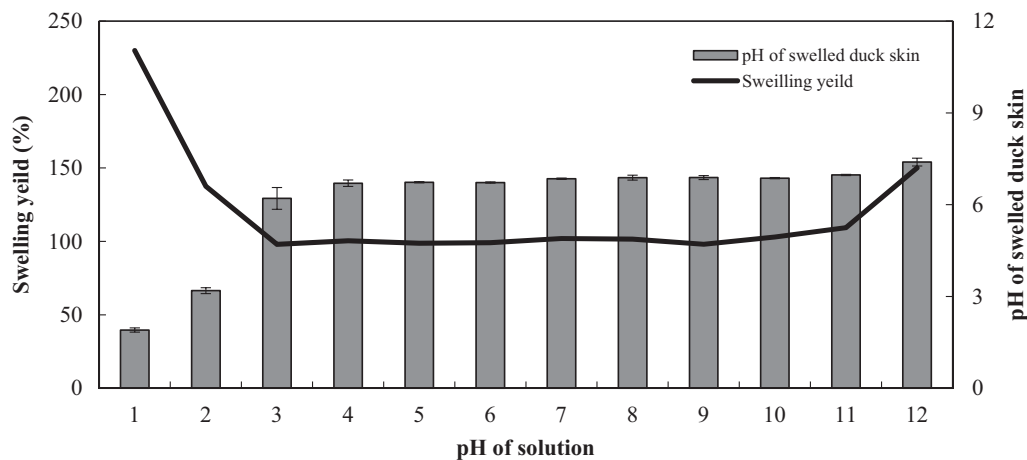


Figure 1. pH after swelling and swelling yield of duck skin with various pH condition.

Transmittance Measurement

The transmittance of the gelatin samples was measured at 600 nm using a spectrophotometer (Libra S22; Biochrom Ltd., England) at 35°C. The light transmitted (expressed as percentage) through the extracted gelatin solution and was calculated as follows:

$$\text{Transmittance (\%)} = 10^{-\text{transmittance at } 600 \text{ nm}} \times 100$$

Protein Quantification And Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

The protein concentration was analyzed using Bradford reagent (Sigma-Aldrich, St. Louis, MO, USA) according to the method of Bradford (1976). The gelatin samples were evaluated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970). Gelatin samples (0.67%) and Laemmli sample buffer (Bio-Rad Lab, Inc., USA) were mixed in the following proportions: 1:2, 1:1, and 3:1. After heated mixed samples to 100°C (5 min), 15 μ L of each sample was injected into the wells of a 12% Mini-PROTEAN TGX Precast Gel (Bio-Rad Lab, Inc., USA). 0.025 M Tris-HCl, 0.250 M glycine, and 0.1% SDS were used as buffer solution. Coomassie Brilliant Blue R250 (B7920; Sigma, USA) was used to stain loaded gel. The separated proteins were identified using the standard protein markers (Precision Plus Protein Standards, Catalog number 1,610,374; Bio-Rad Lab., USA).

Statistical Analysis

Gelatin sample per extraction method was extracted five times and used as experimental units. Statistical analyses were performed using the general linear model (GLM) in SPSS version 19.0. Duncan's multiple range test ($P < 0.05$) was used to determine the difference among the extraction methods.

RESULTS AND DISCUSSION

Rate of Swelling of Duck Skin in The Soaking Solution of Varying pH

The gelatin manufacturing process typically comprises the following 3 main stages: 1. Preconditioning of material such as washing and swelling. 2. Extraction of gelatin from material. 3. Purification or drying of the extracted gelatin (Park et al., 2013). Material pretreatment involves preparing native collagen for gelatin extraction using different heating methods. Campbell and Kenney (1994) reported that acidic or alkali condition facilitated swelling and collagen solubilization due to a disruption the non-covalent bonds in collagen and the protein structure (Park et al., 2013). Thus, the swelling process substantially influences the extraction yield. The rate of swelling of duck skin in soaking solutions of different pH observed in the present study is shown in Figure 1. The rate of swelling followed a U-shaped curve and was the highest at pH 1. The rate of swelling at pH 3 to 11 showed no significant difference, and it ranged from 102 to 104%. At pH 1 to 3, the rate of swelling increased noticeably with decrease in pH, whereas at pH 11 to 13, the rate of swelling increased with increase in pH. This phenomenon can be attributed to different isoelectric points of collagen—type A collagen (isoelectric point pH 8 to 9) and type B collagen (isoelectric point pH 4 to 5) (Hinterwaldner, 1977). Because the duck skin is mainly composed of type A rather than type B collagen (Park et al., 2013), the rate of swelling under acidic condition (pH 1) was higher than that under alkaline condition. In the present study, for gelatin extraction, the skin samples soaked in a pH 1 solution, as it presented the highest rate of swelling. Our results are in agreement with those of Shin (2002), who reported that swelling of pork skin was optimum under acidic condition (pH 2.6, 12 h). Furthermore, Liu et al. (2001) evaluated swelling of chicken with various pH solutions and demonstrated that acidic conditions resulted in optimal swelling.

Table 1. Gelatin extraction yield and gelatin powder yield from duck skin with various heating method.

Parameters	Water bath ¹	Sonicator	Superheated steam	Microwave oven
Gelatin extraction yield (%)	11.71 ± 1.02 ^c	26.15 ± 2.51 ^b	44.02 ± 0.76 ^a	28.51 ± 3.95 ^b
Gelatin powder yield (%)	2.27 ± 0.26 ^b	3.52 ± 0.12 ^a	3.67 ± 0.89 ^a	2.05 ± 0.04 ^b

All values are mean ± standard deviation of 3 replicates.

^{a-c}Means within a row with different letters are significantly different ($P < 0.05$).

¹Water bath: heating for 10 min at 60°C in water bath, sonicator: heating for 10 min at 60°C and 40 kHz in sonicator, steam: heating for 10 min at 120°C in steamer, superheated steam: heating for 10 min at oven 150°C and steam 150°C, microwave: heating for 10 min at 200 W.

Table 2. pH, melting point, gel strength and viscosity of gelatin from duck skin with various heating method.

Parameters	Water bath ¹	Sonicator	Superheated steam	Microwave oven
pH	3.07 ± 0.02 ^a	3.05 ± 0.02 ^b	3.04 ± 0.02 ^{b,c}	3.02 ± 0.02 ^c
Melting point (°C)	33.88 ± 0.25 ^a	33.25 ± 0.65 ^b	31.25 ± 0.29 ^c	32.75 ± 0.29 ^b
Gel strength (kg)	0.25 ± 0.02 ^a	0.22 ± 0.01 ^b	0.21 ± 0.01 ^b	0.26 ± 0.02 ^a
Viscosity (mPa*s)	56.92 ± 6.01 ^c	65.33 ± 1.52 ^b	74.89 ± 3.91 ^a	77.86 ± 3.64 ^a

All values are mean ± standard deviation of three replicates.

^{a-c}Means within a row with different letters are significantly different ($P < 0.05$).

¹Water bath: heating for 10 min at 60°C in water bath, sonicator: heating for 10 min at 60°C and 40 kHz in sonicator, steam: heating for 10 min at 120°C in steamer, superheated steam: heating for 10 min at oven 150°C and steam 150°C, microwave: heating for 10 min at 200 W.

Gelatin Extraction Yield and Gelatin Powder Yield from Duck Skin

The extraction yield is an important factor considered in the assessment of optimal conditions for large-scale industrial production of gelatin from duck production by-products (Park et al., 2013). The gelatin extraction yield from duck skin obtained in the present study is shown in Table 1. Park et al. (2013) reported that heating at a high temperature and efficient heat transfer can increase the extraction yield of collagen. The gelatin extraction yield was the highest with the superheated steam extraction method and the lowest with the water bath extraction method. Generally, gelatin extraction involves heat treatment as it disrupts the hydrogen bonds in the collagen molecule, interfering irreversibly with the three-dimensional structure by solubilizing collagen to form gelatin (Du et al., 2013; Gómez-Guillen et al., 2005).

In the present study, the gelatin powder yield from duck skin (Table 1) with sonication and superheated steam extraction methods was higher than that with the water bath extraction method ($P < 0.01$). The result is in agreement with that of Park et al. (2013), who reported that the gelatin powder yield from duck feet using different treatments ranged from 0.75 to 3.31% and that the yield achieved using an electric pressure cooker was the highest. They also suggested that the combination of high temperature and pressure facilitated efficient extraction of gelatin. In general, the gelatin powder yield represents the amount of dehydrated gelatin obtained from the raw material or the relative ratio of dehydrated gelatin.

pH, Melting Point, Gel Strength, and Viscosity of Gelatin Extracted from Duck Skin

The pH, melting point, gel strength, and viscosity of gelatin extracted from duck skin are presented in Table 2. The pH of gelatin extracted from duck skin using the water bath extraction method was higher than using the other methods ($P < 0.05$). Similarly, Park et al. (2013) reported that the pH of duck feet gelatin extracted using water bath was higher than that of gelatin extracted using other methods. In the present study, the melting point of gelatin extracted using different heating methods ranged from 31.25 to 33.88°C, and gelatin extracted using the superheated steam extraction method presented the lowest ($P < 0.05$) melting point compared with that of gelatin extracted using the other methods. Gómez-Guillen et al. (2005) reported that the melting point of gelatin is directly proportional to its molecular weight. Furthermore, Park et al. (2013) reported that the melting point of gelatin extracted using different methods ranged from 33.06 to 39.38°C, which is similar to that observed in the present study. Haug et al. (2004) suggested that the melting point of gelatin is affected by the proportion of proline and hydroxyproline in the raw material. Thus, it seems that gelatin extracted using different heating methods might have different molecular weights depending on the heating rate, and this can be attributed to the different melting points of gelatin. In the present study, the gel strength of gelatin extracted from duck skin was higher with the water bath and microwave extraction methods compared with

Table 3. Color and transmittance of gelatin from duck skin with various heating method.

Parameters	Water bath ¹	Sonicator	Superheated steam	Microwave oven
CIE L*	20.50 ± 0.76 ^c	42.56 ± 0.35 ^a	16.60 ± 0.04 ^d	28.42 ± 5.64 ^b
CIE a*	-0.25 ± 0.13 ^a	-0.94 ± 0.01 ^c	-0.16 ± 0.08 ^a	-0.51 ± 0.17 ^b
CIE b*	-1.51 ± 0.18 ^a	-1.48 ± 0.03 ^a	-2.20 ± 0.01 ^b	-2.02 ± 0.31 ^b
Transmittance (%)	36.90 ± 4.54 ^a	2.35 ± 0.34 ^c	35.74 ± 3.52 ^a	15.71 ± 1.10 ^b

All values are mean ± standard deviation of three replicates.

^{a-d}Means within a row with different letters are significantly different ($P < 0.05$).

¹Water bath: heating for 10 min at 60°C in water bath, sonicator: heating for 10 min at 60°C and 40 kHz in sonicator, steam: heating for 10 min at 120°C in steamer, superheated steam: heating for 10 min at oven 150°C and steam 150°C, microwave: heating for 10 min at 200 W.

that of gelatin extracted with the other methods ($P < 0.05$). According to Park et al. (2013), the gel strength might be associated with the degree of protein degradation due to swelling and various heating processes. Choi and Regenstein (2000) reported that gel strength is related to the melting point and that it would normally increase with the melting point of gelatin. Moreover, Ahmad et al. (2010) reported that gel strength is influenced by the proportion of proline and hydroxyproline in the raw materials. Moreover, the gel strength is directly proportional to the gelatin concentration (Choi and Regenstein, 2000). In the present study, the viscosity of gelatin extracted from duck skin using the superheated steam and microwave extraction methods was higher than that of gelatin extracted using the water bath and sonication extraction methods ($P < 0.05$). Park et al. (2013) reported that the viscosity of gelatin reveals the specific hydrodynamic volume of gelatin in the liquid phase. Badii and Howell (2006) reported that gelatin with a high viscosity is commercially valuable for application in food industry. Karim and Bhat (2009) reported that the melting point, gel strength, and viscosity are the most important physical properties of gelatin determining its application. The physical properties of gelatin depend not only on the source, but also on the extraction method. The physical properties of heat-treated gelatin improved under high processing pressure and high extraction temperature (Montero et al., 2002). In this study, gelatin extracted using the superheated steam extraction method presented the lowest melting point and gel strength, and the highest viscosity ($P < 0.05$). Low melting point of gelatin is not proper to mouth feeling and preference of consumer because lower viscosity perception brings the negative texture of food (Choi and Regenstein, 2000). However, the highest viscosity of gelatin extracted by super-heated steam could prevent the negative effect of a lower melting point than another extraction method.

Color and Transmittance of Gelatin from Duck Skin

The color and transmittance of gelatin extracted from duck skin are presented in Table 3. The highest CIE L* value was obtained for gelatin extracted using the sonication extraction method, whereas the

lowest was for gelatin extracted using the superheated steam extraction method ($P < 0.05$). Although the redness (CIE a*) and yellowness (CIE b*) of gelatin extracted by different heating methods showed statistical differences, the values were close to zero, indicating the pale appearance of gelatin. In general, the color of gelatin ranges from pale yellow to dark amber (Cole and Roberts, 1997). Ninan et al. (2011) reported that the color of gelatin is significantly affected by the raw material color and extraction process. In the present study, as gelatin was extracted from the same duck skin sample (CIE L*, 65.25; a*, -1.12; b*, 6.37; after swelling), the color of gelatin was significantly affected by the extraction method. The transmittance of gelatin extracted using the water bath and superheated steam extraction methods was the highest, whereas gelatin extracted using the sonication extraction method presented the lowest transmittance ($P < 0.05$). Studies have reported that the dark color and turbidity of gelatin are due to the generation of inorganic, proteinaceous, and mucosubstance contaminants (Eastoe and Leach, 1977) or Maillard reaction (Ahmad and Benjakul, 2011) during the manufacturing process. However, the color of gelatin has negligible effect on its functional properties (Ockerman and Hansen, 1988).

Protein Quantification and SDS-PAGE of Gelatin Extracted from Duck Skin

The results of protein quantification and SDS-PAGE of samples extracted from duck skin using various extraction methods are shown in Figure 2. The proportion of decomposed gelatin in samples can be determined using the molecular weight of proteins in the samples determined by SDS-PAGE (Park et al., 2013). The samples obtained using superheated steam (6117.24 $\mu\text{g/mL}$) and microwave extraction methods (5923.97 $\mu\text{g/mL}$) had higher protein concentration than that of samples obtained using water bath (4636.19 $\mu\text{g/mL}$) and sonication extraction methods (4825.23 $\mu\text{g/mL}$) (Figure 2A), as confirmed by the extraction yield (Table 1). The results indicate that gelatin extracted from duck skin was highly decomposed by the superheated steam extraction method compared with that by the other methods, owing to the low molecular weight of gelatin (Montero et al., 2002; Park et al., 2013). Figure 2B shows the SDS-PAGE

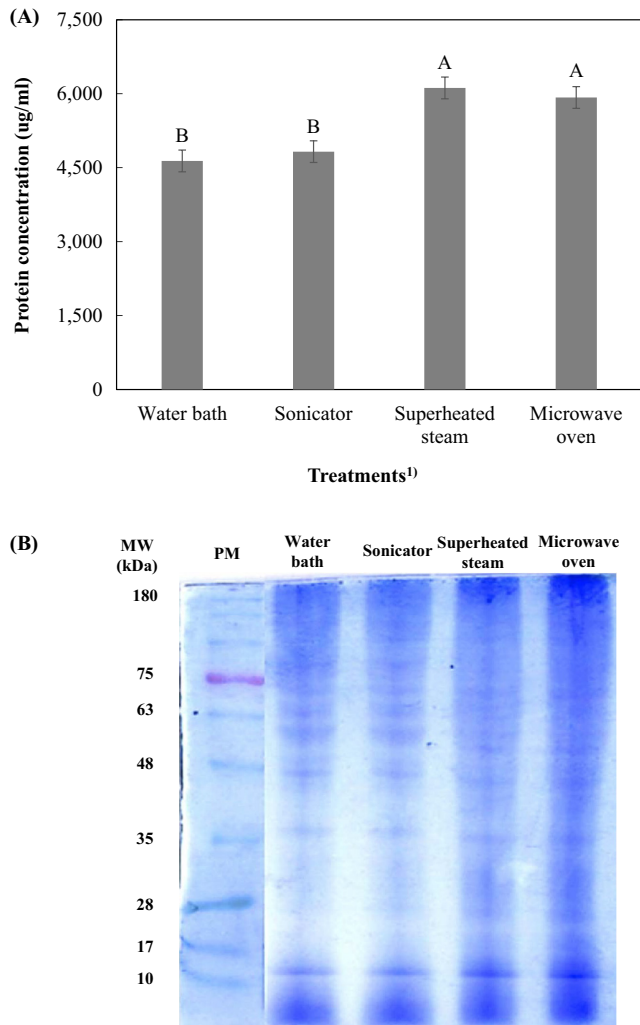


Figure 2. The protein quantitation and SDS-PAGE of protein fraction in duck skin with various extract conditions. MW = molecular weight; PM = protein marker. (A) protein concentration and (B) SDS-PAGE of protein fraction. Values with different letters are significant ($P < 0.05$). ¹⁾Water bath: heating for 10 min at 60°C in water bath, sonicator: heating for 10 min at 60°C, and 40 kHz in sonicator, steam: heating for 10 min at 120°C in steamer, superheated steam: heating for 10 min at oven 150°C, and steam 150°C, microwave: heating for 10 min at 200 W.

patterns of gelatin extracted using the different extraction methods. The samples obtained using the superheated steam and microwave extraction methods presented more intense bands (high relative staining intensity) than those of samples obtained using the water bath and sonication extraction methods. This indicates that samples obtained using the superheated steam and microwave extraction methods contained a higher amount of completely decomposed gelatin than that of the samples obtained using the other methods. It has been reported that the microwave extraction method is more efficient than the conventional water bath extraction method in extracting soluble soy proteins (Choi et al., 2006). Additionally, the superheated steam extraction method could be an efficient method for extracting gelatin from fruits and plants (Basile et al., 1998; Wang et al., 2018). Thus, superheated steam and microwave

extraction methods are more efficient than the other extraction methods for gelatin extraction.

CONCLUSIONS

We evaluated the physicochemical properties of gelatin extracted from duck skin using the water bath, sonication, superheated steam, and microwave extraction methods. Compared to the other methods, the superheated steam extraction method presented a better extraction yield, gelatin powder yield, melting point, gel strength, and gelatin viscosity. Furthermore, gelatin extracted using this method presented the optimal physicochemical properties. Thus, we conclude that superheated steam extraction is the best method for extracting gelatin from duck skin for use in meat products.

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