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Extraction and characterization of gelatin from chicken feet by acid and ultrasound assisted extraction

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Abstract

The objective of this study was to extract gelatin from chicken feet using 2 different methods (acid extraction and ultrasound assisted extraction). The obtained gelatin was compared with commercial bovine gelatin in terms of gelatin powder and gelatin gel. Various properties such as, yield, pH, color (L*, a* and b*), proximate analysis (moisture, protein, fat and ash), Fourier Transform Infrared (FTIR) spectrum, maximum force and electrophoresis of gelatin were evaluated. The highest yield of chicken feet gelatin was obtained from acid extraction method with 4.05% (wet weight or 12.64% based on dry weight). Proximate analysis of chicken feet gelatin showed the acid extraction has the higher protein content than ultrasound assisted extraction with 90.06 ± 1.43, while ultrasound assisted gelatin has the lowest moisture content with 5.40 ± 2.44. However, there is no significantly different values of protein, fat, ash and moisture content between acid, ultrasound extraction and commercial bovine gelatin ($p>0.05$). pH of gelatin solution for both methods has range between 6.13-6.49. Amide A peak was shown at the wave numbers 3,322.12 cm^{-1} for gelatin extracted with acid condition and 3,619.04 cm^{-1} for commercial bovine gelatin.

Keywords: chicken feet, gelatin, physico-chemical properties, ultrasonic-assisted extraction

1. Introduction

Gelatin is one of the most common food additives that used in foods, obtained from denaturation of collagen and have many applications in food and non-food industries. In food industry, gelatin is one of the water soluble polymers that can be used to improve stability and consistency of food. While in non-food industry such as medical and pharmaceutical, it can be used to produce soft and hard capsules, wound dressing, and adsorbent pads. Generally, the sources of gelatin produced from bovine and pig skins and demineralized bones and hooves (Baziwane and He, 2003). In the mid-1990s, the world-wide demand for gelatin has been increasing. According to transparency market research (Sheela, 2013), the global demand of gelatin for

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food and non-food applications has reach 348.9 kilo tons in 2011 and will be expected to reach 450.7 kilo tons in 2018 where 40% of the overall production in 2011 was utilized from pig skin.

For several reasons there are still serious concern among the consumers to consume gelatin which produce from bovine and porcine bones and skins. This is because some problems such as religious matter, mad cow disease (Bovine Spongiform Encephalophy: BSE) and social reasons (Karim and Bhat, 2009). Thus, lead to many researchers to discover alternatives sources such as fish (marine and freshwater) and poultry. Gelatin from poultry by-products have also been receiving some attention since the wastes (blood, viscera, feet, bone, mechanically deboned and feather) generated during processing contains varying amount of protein where head, feet and skin are rich in collagenous protein (Lasekan et al., 2013).

Extraction of gelatin from chicken feet has been done by using alkaline treatment where the higher extraction percentage yield of chicken feet gelatin powder was obtained at 18% w/w (Rahman and Jamalulail, 2012). While Sarbon et al. (2013) reported that 16% gelatin was obtained from chicken skin by using acid treatment. The acid treatment usually used for extract gelatin or collagen from young animal such as pig, fish and poultry within short period between 10 to 48 h. While alkaline treatment frequently used for mature animal with complex cross structure, such as bones and cartilage cattle and buffalo which takes long period to extract the gelatin from 6-20 days (Rahman and Jamalulail, 2012). According to Hao et al. (2009) the quality of gelatin depends on its physical properties, which is influences by both species and tissue from which it is extracted and from the extraction method. Therefore, this study was conducted to investigate the gelatin extraction and its physicochemical properties by using different extraction methods (acid and ultrasonic assisted extraction) and to compare with commercial bovine gelatin.

2. Materials and Methods

2.1 Raw materials

Glycerol and other analytical grade reagents were obtained from Merck (Darmstadt, Germany). Electrophoresis reagents were obtained from Bio-Rad Laboratories (Hercules, CA, USA). The commercial bovine gelatin type B (240 blooms) was obtained from Gelita NZ Limited (Woolston Christchurch, New Zealand). Chicken feet were purchased from Bandu local market (Chiang Rai, Thailand).

The frozen chicken feet was thawed at 4°C for 20 h (Niu et al., 2013) and cleaned in running tap water before segmenting into small pieces (0.5×0.5 cm). The bone was removed first and the remaining part was used as a starting material for gelatin extraction.

2.2 Gelatin extractions

2.2.1 Acidic extraction method

The extraction procedure was conducted according to Irwandi et al. (2009) with slight modification. To extract gelatin, the small pieces of chicken feet was soaked into sodium hydroxide with concentration 0.2% (w/v) to remove non-collageneous material. The mixture was shaken and stirred at room temperature (22-28°C) for 40 min. The alkaline solution was repeated three times. During alkaline treatment, the undesirable components were removed and texture of the material became soft and ready for the gelatin extraction. Then samples was soaked in acetic acid 0.2% (v/v) for 40 min for further extraction, then the acid solution was drained and washed with running tap water until pH neutral and the final extraction of gelatin was performed in distilled water at 70°C for 90 min at the skin water ratio of 1:9 (w/v). The extract was then filtered through two layers of cheese clothes and freeze dried. The dried matter was grounded as gelatin powder.

2.2.2 Ultrasound assisted extraction method

Extraction conditions by using ultrasound assisted extraction (UAE) of gelatin were based on Ming (2013) with slightly modification. The acid treated chicken feet were extracted by using temperature70°C and ultrasonic power 300 W for 100 min. The extract was then filtered through two layers of cheese clothes and freeze dried. The dried matter obtained was ground as gelatin powder.

The procedure for extracting gelatin was essentially based on a mild acid pretreatment for collagen swelling due to sufficient to produce adequate swelling and to disrupt the non-covalent, intra- and inter-molecular bond, followed by extraction in water at moderate temperature (above 40°C) to destroy hydrogen bonding which stabilizes helix to coil transition and result in conversion to soluble gelatin (Karim and Bhat, 2009).

2.3 Determinations of gelatin properties

2.3.1 Yield

The yield of gelatin was calculated based on wet weight of fresh skin (Balti, 2011) and dry weight (Kaewruang, 2013) by using the following equation:

Yield of wet weight (%) = [weight of freeze dried gelatin (g) / wet weight of fresh skin (g)] × 100

Yield of dry weight (%) = [weight of dry gelatin (g) / weight of initial dry skin (g)] × 100

2.3.2 Proximate composition

The moisture, ash and fat contents of the gelatin and raw materials was determined according to the AOAC methods number 927.05, 942.05, and 920.38B, respectively (AOAC, 2000). The protein content was determined by estimating its total nitrogen content by Kjeldahl method according to the AOAC method number 984.13 (AOAC, 2000).

2.3.3 Color

The color of gelatin gels was measured by using the color meter. *L**, *a** and *b** indicated lightness/brightness, redness/greenness and yellowness/blueness, respectively, was recorded. The colorimeter will warm up for 10 min and calibrated with a white standard.

2.3.4 pH

The pH of gelatin solution was determined by using the British Standard Institution method, BSI 757 (1975) where 1% (w/v) gelatin solution was prepared in distilled water and cool to 25^{o}C in a water bath. The pH was measured by using pH meter (Eutech/cyberscan PH510) with a glass electrode after standardizing with 4 and 7 pH buffers.

2.3.5 Fourier transform infrared spectra analysis

For FTIR spectra analysis, freeze-dried gelatin samples was placed on the crystal cell and the cell could be clamped into the mount of the FTIR spectrometer. The spectra in range 400-4000 cm^{-1} was ratio and automatic signals gained was collected in 32 scans at a resolution of 4 cm^{-1} against the background spectrum recorded from the clean empty cell at 25^{o}C (Ahmad and Benjakul, 2011).

2.3.6 Electrophoretic analysis

Protein patterns of gelatin sample were determined by SDS-polyacrylamide gel electropheresis (PAGE) according to Sai-ut et al. (2012). The samples (1 g) was dissolve in 10 mL of 5% (*w/v*) SDS solution and then heated at 85°C for 1 h. Supernatants were mixed with sample buffer (0.5 M tris-HCl, pH 6.8 containing 4% (*w/v*) SDS, 20% (*v/v*) glycerol, and 10% (*v/v*) βME) at the ratio of 1:1 (*v/v*). The mixture was boiled for 3 min. Protein samples (15 μg) was loaded into the polyacrylamide gel made with a 7.5% (*v/v*) running gel and 4% (*v/v*) stacking gel and remove to electrophoresis at a constant current of 15 mA per gel using a power pac basic 9Bio-Rad laboratories). After electrophoresis, the gel was stained with 0.1% (*w/v*) Coomassie blue R-250 in 15% (*v/v*) methanol and 5% (*v/v*) acetic acid and destained with 30% (*v/v*) methanol and 10% (*v/v*) acetic acid.

2.4 Preparation of gelatin gel and gel strength determination

Gelatins powders were mixed with distilled water to obtain a final concentration of 6.67% (w/v). The mixture was stirred and kept at room temperature for 30 min to allow the gelatin to swell. Then, 25 mL of the mixtures was transferred into glass beaker (50 mL) and incubated at 40°C for 30 min. The sample was kept in temperature-controlled chamber at 10°C and allowed to stand for 16-18 h before further determination (Jeongjareonrak et al., 2010).

The gel strength of the gelatin gels and commercial bovine gelatin were prepared at 10°C and determined using TA-XT2 Texture Analyzer with a load cell of 5 kN and equipped with 1.27 cm diameter flat-faced cylindrical Teflon. The maximum force (in grams) was recorded when the penetration distance reached 4 mm and the speed of the plunger is 0.5 mm/s (Rahman and Jamalulail, 2012).

2.5 Statistical analysis

The data was subjected to analysis of variance (ANOVA). A mean comparison was carried out by Duncan's Multiple Range Tests. Significance of difference was defined at $P<0.05$. The analysis was performed by using an SPSS package (SPSS 16.0 for window, SPSS Inc, Chicago, IL).

3. Results and Discussion

3.1 Gelatin yield

The yield of extracted gelatin from chicken feet using acid extraction was 4.05% (wet weight basis or 12.64% on dry weight basis), while the using of ultrasonic assisted extraction method has lower yield compare to previous method which has 3.96% based on wet weight (or 12.37% on dry weight basis). The lower yield of the gelatin may due to the loss of extracted collagen through leaching during washing in the pretreatment process or due to the incomplete of hydrolysis of collagen (Sarbon et al., 2013). The differences in gelatin percentage obtained were influenced by species, age of animals, proximate composition,collagen content and methods of the extraction (Songchotikunpan et al., 2008). Alkali and acid treatment was conducted to weaken the collagen structure, solubilize the non-collagen proteins and hydrolyse some of the peptide bonds and keep the consistency of the collagen fibres (Sarbon et al., 2013).

3.2 Proximate analysis of gelatin

The proximate composition of gelatin extracted from chicken feet by using different extraction methods **(**Table 1**)**. Moisture content of gelatin extracted from acid and ultrasound extraction was 6.73% and 5.40%, respectively. In comparison raw materials and commercial bovine gelatin have moisture content 60.66% and 11.75%, respectively. Moisture content may vary due to different treatment of the materials (freezing, drying, scrapping and so on) (Taheri et al., 2009). Low moisture content increases the shelf life of gelatin and can prevent gelatin to be sticky (Rahman and Jamalulail, 2012). The commercial bovine gelatin has the highest protein content with 91.97% followed by gelatin from acid extraction with 90.06%, and 88.35% from ultrasound assisted extraction. The fat content of raw material is higher than extracted gelatin with 2.19%, while from the extracted gelatin of acid extraction has no significant difference result ($p<0.05$) in comparison with ultrasound assisted gelatin with respective values of 1.67% and 0.66%. The ash content of gelatin varies depends on the raw material and the method of processing (GMIA, 2012). Proximate analysis shows that ash content in gelatin from both methods have no significantly different with commercial bovine gelatin and raw materials, where the ash content were in the range 0.18-0.84%. According to Wasswa et al. (2007) the ash content in gelatin powder should not exceed 2%, the low percentage of fat and ash indicated that gelatin processing has done effectively.

Table 1. Proximate analysis of gelatin from chicken feet and commercial bovine gelatin.

Composition (%wt)	Chicken feet	Gelatin		
		Acid extraction	UAE	Commercial
Moisture	60.66 ± 2.44[a]	6.73 ± 2.44[b]	5.40 ± 2.44[b]	11.75 ± 2.44[b]
Protein	18.69 ± 0.92[b]	90.06 ± 1.43[a]	88.35 ± 2.10[a]	91.97 ± 0.07[a]
Fat	2.19 ± 0.70[b]	1.67 ± 0.36[bc]	0.66 ± 0.24[c]	5.09 ± 054[a]
Ash	0.14 ± 0.13[b]	0.18 ± 0.07[ab]	0.38 ± 0.42[ab]	0.84 ± 0.13[a]

[a-c] Different letters in the same row indicate significant difference ($P < 0.05$).

3.3 Color

The color of gelatin obtained from 2 different extraction techniques in comparison with commercial gelatin is shown in Table 2. The big different was observed between the extracted gelatin powder and the commercial gelatin, especially a* and b* values. The highest in lightness value was found in gelatin extracted with UAE.

For gelatin gel, differences in color were observed in (6.67%) gelatin solutions of extracted gelatin (Table 2). Gelatin extracted from acid extraction method showed the higher *L**-value (lightness) than others (UAE and CBG). The redness (*a**-value) for ultrasound assisted extraction and acid extraction were -1.43 ± 0.05 and -1.01 ± 0.44 respectively which is significantly different ($P < 0.05$) with commercial bovine gelatin with -0.42 ± 0.53, while for b*-value, acid extraction shows the reading -2.53 ± 0.82 with commercial bovine gelatin -1.57 ± 0.92. While for the color of the gelatin powder shown that gelatin extracted using ultrasonic extraction has the higher value of L* with 47.97 ± 0.73 (Table 4). Gelatin color is influence by the raw material and not affects the nature and chemical quality of gelatin (Rahman and Jamalulail, 2012).

Table 2. Color of gelatin powder and gelatin gel from chicken feet and commercial bovine gelatin.

Treatment	Color		
	L*	***a****	***b****
Gelatin powder			
Acid extraction	61.45 ± 2.82[c]	-0.36 ± 0.02[b]	8.49 ± 0.20[c]
Ultrasound assisted extraction	72.91 ± 0.66[a]	-0.10 ± 0.11[c]	4.42 ± 0.12[b]
Commercial bovine gelatin	69.70 ± 0.56[b]	2.36 ± 0.10[a]	21.38 ± 0.12[a]
Gelatin gel			
Acid extraction	47.97 ± 0.73[a]	-1.01 ± 0.44[b]	-2.53 ± 0.82[a]
Ultrasound assisted extraction	44.95 ± 0.61[b]	-1.43 ± 0.05[b]	-4.92 ± 2.39[b]
Commercial bovine gelatin	29.29 ± 0.32[c]	-0.42 ± 0.53[a]	-1.57 ± 0.92[a]

[a-c] Different letters in the same row indicate significant difference ($P < 0.05$).

3.4 pH and gel strength

The pH value of the extracted gelatin from chicken feet using acid extraction method was higher than ultrasound assisted extraction and commercial bovine gelatin with the respective values 6.49 ± 0.49, 6.13 ± 0.00 and 5.03 ± 0.03 (Table 3). Previous studies on the pH values of fish gelatin showed lower values than chicken feet gelatin such as sin croaker (pH 3.35), shortfin scad (pH 4.87) (Cheow et al., 2007), rohu (pH 4.08), and common carp (pH 4.05) (Ninan et al., 2010). The pH value of gelatin is influenced by the type and strength of the chemical that used during extraction procedure (Songchotikunpan et al., 2008).

Table 3. pH and maximum force of gelatin gel from chicken feet and commercial bovine gelatin.

Treatment	pH	Max force (g)
Acid extraction	6.49 ± 0.49[a]	1.85×10^{2} ± 8.13[a]
Ultrasound Assisted extraction	6.13 ± 0.00[b]	79.23 ± 11.59[b]
Commercial bovine gelatin	5.03 ± 0.03[c]	94.56 ± 12.05[b]

[a-c] Different letters in the same row indicate significant difference ($P < 0.05$).

The maximum forces of gelatin gels were measured in grams by using a plate to put pressure on the surface of the gel (Schrieber and Gareis, 2007). Gelatin from acid extraction has the higher value of maximum force with 1.85×10^{2} ± 8.13 compared with commercial bovine

gelatin and ultrasound assisted extraction gelatin (Table 4). The difference in the maximum force value was directly influences by the extraction conditions (Kaewruang et al, 2013) and intrinsic properties such as the composition of the protein (molecular weight distribution) (Karim and Bhat, 2008).

3.5 Fourier Transform Infrared Spectra (FTIR) analysis

FTIR spectroscopy has been used to monitor the functional group and secondary structure of the gelatin (Kaewruang et al., 2013). Fig. 1 shows the FTIR spectra of the extracted gelatin from chicken feet and commercial bovine gelatin. FTIR spectra of the extracted gelatin from chicken feet show the major peaks in the amide region. Chicken feet gelatin from acid extraction showed the vibration peak at the wave numbers 1,658.74 cm^{-1} to the amide I, 1,552.44 cm^{-1} to the amide II, 1,236.36 cm^{-1} to the amide III, 2,924.36 cm^{-1} to the amide B and 3,322.12 cm^{-1} to the amide A. Extracted gelatin from ultrasound assisted extraction exhibited FTIR spectra that not significantly different from acid extraction gelatin. The FTIR spectra of commercial bovine gelatin amide I, II, and A were noticeable at 1,695.10 cm^{-1}, 1,530.06 cm^{-1} and 3,619.04 cm^{-1}, respectively

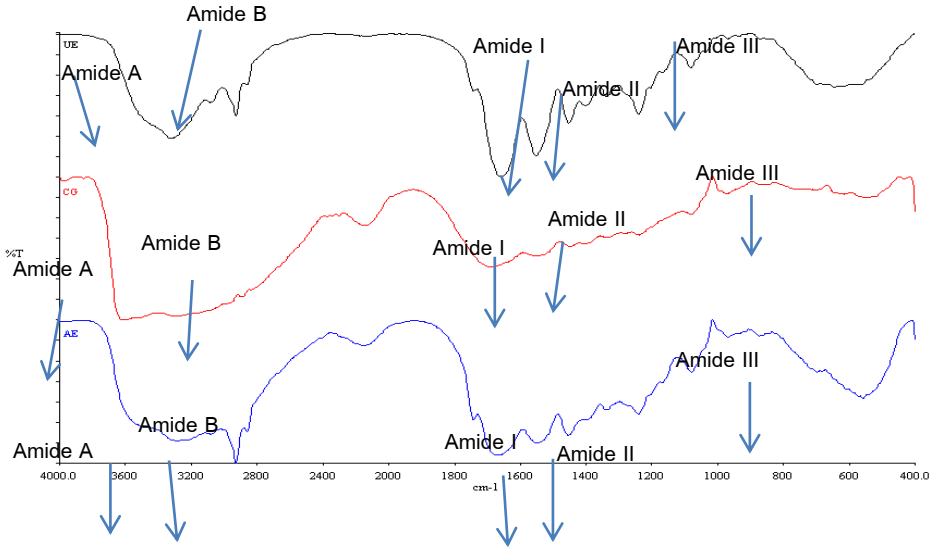

Figure 1. FTIR of gelatin from chicken feet and commercial bovine gelatin (UE: Ultrasound Assisted Extraction Gelatin, CG: Commercial Gelatin, and AE: Acid Extraction Gelatin).

Almeida et al. (2012) reported that gelatin from chicken feet by acid extraction has the amide I at 1,652.01 cm^{-1}, amide II at 1,539.87 cm^{-1}, amide III at 1,241.29 cm^{-1}, amide B 2,932.72 at cm^{-1} and amide A at 3,399.56 cm^{-1} which are no significantly different with this study. The absorption in amide I is a C=O stretching/hydrogen vibration coupled with COO^-.The amide II vibration mode is the combination of CN stretch and in-plane NH deformation mode of the peptide group. The amide III represented the combination peaks between C-N stretching vibrations and N-H deformation from linkages as well as absorptions arising from wagging vibrations from CH_2 groups. The amide A also tends to join with CH_2 stretch peak, while the amide B suggests the interaction of $–NH_3$ group between peptide chains (Almeida et al., 2012).

3.6 Electrophoretic analysis

Protein pattern of extracted gelatin from 2 different methods is shown in Fig. 2. The molecular weight of chicken feet gelatin compare with the commercial bovine gelatin was discussed. The extracted gelatin from acid extraction showed the major protein band with the molecular weight of 198 kDa and 130 kDa from both extraction methods.

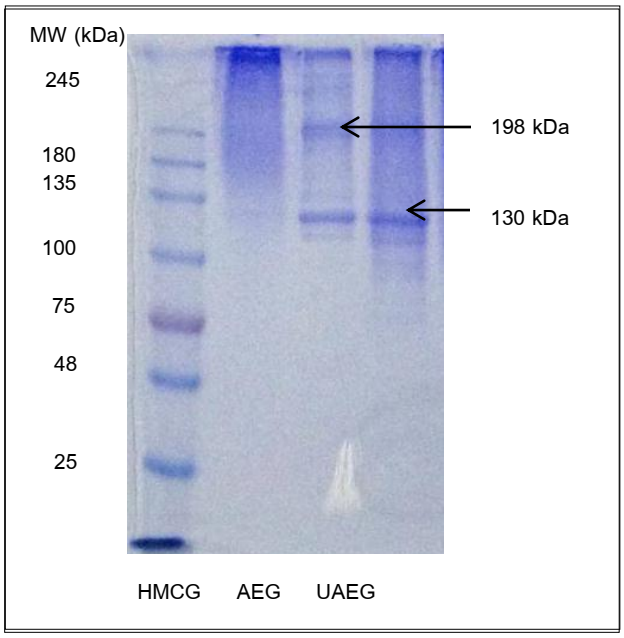

Figure 2. SDS-PAGE of gelatin from chicken feet. HM: MW markers, CG: commercial gelatin, AEG: Acid extraction gelatin and UAEG: Ultrasound assisted extraction gelatin.

However, the commercial bovine gelatin has no identified major protein bands exhibited. Molecular weight of the extracted gelatin may be affected by the hydrolysis process that contributes to the splitting of the peptide chains (Sarbon et al., 2013). During gelatin extraction, the conversion of collagen to gelatin with varying molecular mass took place due to cleavage of interchain cross-links (Kaewruang, 2013).

4. Conclusion

Gelatin from chicken feet was successfully extracted using acid and ultrasound assisted extraction, even though the yield is relatively low. However, the properties of extracted gelatin from both methods have no significantly different compare with commercial bovine gelatin. Although the different extraction processes can influences the properties of gelatins obtained, results from this study indicated that gelatin from chicken feet can be proposed as an alternative gelatin.

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