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Gelatin from chicken feet: papain-assisted extraction, characterization and its application

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ABSTRACT

The characteristics and physico-chemical properties of gelatin from chicken feet were investigated and compared to the commercial bovine gelatin (CBG). The chicken feet gelatin was extracted by the presence of crude papain (1%, w/w) for 12 h at 37°C. The gelatin yield was 18.4% based on dry weight basis. The obtained gelatin have protein content lower than CBG with 40.06%, as well as for moisture and fat with 7.56% and 1.13%, respectively. Extracted gelatin showed viscosity of 2.98 Cp, setting point at 20°C, and melting point at 30°C, which is similar to CBG (30°C). For Fourier Transform Infrared (FTIR) spectrum, chicken feet gelatin has almost similar peak of amide I, II, III, A and B with CBG, with 1,745.62 cm^{-1} to amide I, 1,553.19 cm^{-1} to amide II, 1,238.51 cm^{-1} to amide III, 2,926.42 cm^{-1} to amide B, and 3,322.84 cm^{-1} to amide A. Chicken feet gelatin exhibited similar fat binding capacity, foaming and emulsifying properties with CBG, while slightly different for water holding capacity ($P<0.05$). Furthermore, the major components of extracted gelatin had the molecular weight of 130 and 143 kDa. Gelatin obtained by papain-assisted extraction showed capability as juice clarifier as indicated by lowering the guava juice turbidity. For instance, at the same concentration applied (0.16%), the turbidity of the gelatin extracted was 204 FTU, while CBG was 367 FTU.

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INTRODUCTION

Gelatin is a substantially pure protein ingredient obtained from degradation of collagen which is commonly found in animal skin, bones and connective tissue. There are two types of gelatin. Type A is derived by using acid pretreatment and type B is obtained from alkaline pretreatment (Baziwane and He, 2003). So far, the main sources to produce gelatin were obtained from porcine and cow skins and bones. However those sources lead to some interest among the consumer who concern about halal and kosher market and mad cow disease. As a result, researchers and industry have been trying to develop alternative source (Karim and Bhat, 2008).

The application of gelatin has been used in food industry and non-food industry. For both industries, chemical, physicochemical and functional properties are important. However, for food industry, the main properties characterizing are viscosity, melting point, setting point, and gel strength. Those properties are affected by various causes, such as molecular weight distribution, pH, gel maturation time and temperature. The lower of molecular weight could be occurred if there is cleavage of inter-chain covalent crosslinks and unfavorable breakage of intra-chain peptide (Karim and Bhat, 2009). Gelatin has been traditionally used to clarify wine, beer and fruit juices. It works as clarifying agent by sticking to the particles, or by using charged ions to cause particles to stick each other. The colloidal particle that formed over period of time may precipitate, resulting transparent or clear juice (Benitez and Lozano, 2007).

Gelatin from poultry by-product has 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). Sarbon *et al*. (2013) reported that 16% gelatin was obtained from chicken skin by using acid extraction. While Rahman and Jamalulail, (2012) has been extracted gelatin from chicken feet by using alkaline treatment where the higher extraction percentage yield of chicken feet gelatin powder was obtained at 18% w/w. Papaya latex was used for preparing protein hydolysates since its rich cysteine endopeptidases, such as papain, glycyl endopeptidase, chymopapain, and caricain. Due to the abundance of glycine in gelatin molecules, glycyl endopeptidase as major constituent (30% of total protein) can severe as a potential protease which preferably cleaves the peptide bonds in gelatin (Karnjanapratum and Benjakul, 2014).

Extraction of gelatin from chicken feet by using various methods has been reported (alkaline and acid extraction). However, there is no report using papain assisted extraction technique. Therefore, this study was conducted to investigate the gelatin extraction, physicochemical properties and its application by using papain assisted extraction in comparison with commercial bovine gelatin.

MATERIALS AND METHODS

Raw material and preparation

Chicken feet were purchased from local market (Chiang Rai, Thailand). 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).

The frozen chicken feet were thawed at 4 °C for 20 h and cleaned in running tap water before segmenting into small pieces. The bone was removed first and the remaining part was used as a starting material for gelatin extraction.

Enzyme preparation

Papain was obtained from papaya latex, where the latex was mixed with distilled water (1:1, v/v) and centrifuged at 8000 g at 4°C for 10 min. The supernatant was referred as crude papain extract (Rawdkuen *et al.*, 2010).

Gelatin extraction

Deboned chicken feet were soaked into 0.2% sodium hydroxide at pH 13 for 2 h at 37°C and then soaked into 5% acetic acid at 37°C for 2 h for further extraction. 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 with 1% w/w of crude papain extract at 37°C for 12 h. The extract then filtered through two layers of cheese clothes and freeze dried. The dried matter was ground as gelatin powder.

Determinations of gelatin properties

Yield

The yield of gelatin was calculated based on wet weight and dry weight basis by using the following equation:

$$\text{Yield of wet wight (\%)} = \frac{\text{[weight of freeze dried gelatin (g)]}}{\text{wet weight of fresh skin (g)}} \times 100$$

$$\text{Yield of dry weight (\%)} = \frac{\text{[weight of dry gelatin (g)]} \times 100}{\text{weight of initial dry skin (g)]}}$$

Proximate composition

The moisture, ash and fat contents of the extracted gelatin were 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).

Color

The color of extracted gelatin was measured by using the color meter (Color Quest XE, Hunter Lab, Virginia). *L**, *a** and *b** indicated lightness/brightness, redness/greenness and yellowness/blueness, respectively, was recorded. The colorimeter was warmed up for 10 min and calibrated with a white standard.

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°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.

Fourier transforms infrared spectra (FTIR) analysis

For FTIR spectra analysis, freeze-dried gelatin was placed on the crystal cell and the cell could be clamped into the mount of the FTIR spectrometer (FTIR spectrum GX, Perkin Elmer, USA). 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°C (Ahmad and Benjakul, 2011).

Electrophoretic analysis

Protein patterns of gelatin were determined by SDS-PAGE according to Saiut *et al*., (2012). The sample (1 g) was dissolved in 10 mL of 5% (*w/v*) SDS solution and then heated at 85°C for 1 h. Supernatant was 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 sample (15 µg) was loaded into the polyacrylamide gel made with a 7.5% (*v/v*) running gel and 4% (*v/v*) stacking gel and moved to electrophoresis at a constant current of 15 mA per gel using a power pac basic Bio-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.

Viscosity

The viscosity of gelatin solution (6.67%, w/v) was determined with a viscometer (Model LVDV-III, Brookfield Engineering Laboratories, Inc, Middleboro, MA) at 60°C. The speed of the spindle was adjusted to 20 rpm by using 16 mL of gelatin solution (Niu *et al*., 2013).

Setting point and melting point

Setting point and melting point were determined by using 30 mL of gelatin solution (10% ,w/v), where prepared in warmed water bath at 40°C. For setting point, the gelatin solution was putted at cooling water bath by slowly addition of iced-water for time intervals 15s. Thermometer was put to the solution and out each 15s until any drop does not drip, this temperature was recorded as gelatin "setting point". While for melting point, the gelatin solution was putted to the refrigerator at 7°C for 16-18 h and transferred to the water bath at 10°C with gradually addition of warm water at 45°C and melting temperature was recorded (Tavakolipour, 2011).

Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) of gelatin were determined using homogenizer (model IKA/T10, Betchai Bangkok equipment and chemical co. Ltd). Emulsion was prepared by using soybean oil (10 mL) and added to 6 mL of gelatin solution (protein concentration 2%, w/v) and then mixture it with the homogenizer at 20,000 rpm for 1 min at room temperature (26-28°C). Aliquot of the emulsion (50 µL) were taken at the bottom of the container at 0 and 10 min and diluted 100-fold with 0.1% SDS solution (Balti *et al.*, 2011). The absorbance was measured immediately (A_0) and 10 min (A_{10}) after emulsification at 500 nm. EAI and ESI will calculate using the following equation:

$$EAI\ (m^2/g) = (2\ 2.303\ A\ DF)/1\emptyset\ C$$

$$ESI = A_0\ \Delta_t / \Delta_A$$

Where $A = A_{500}$, DF = dilution factor, 1= path length of cuvette in centimeter, $\emptyset$= oil phase volume fraction (0.25), C = the protein content in g/m3, $\Delta A = A_0 - A_{10}$, and Δt = 10 min (Kittiphattanabawon *et al*, 2010).

Foaming properties

Foam expansion (FE) and foam stability (FS) of gelatin were determined by the method of Kittiphattanabawon *et al*. (2010). The gelatin solution (10 mL) with a protein concentration of 2% (w/v) was transferred into 50 mL-Cylinders and then homogenized for 1 min using homogenizer (model IKA/T10, Betchai Bangkok equipment and chemical co.ltd) at 16,000 rpm. The mixtures then incubated for 0, 30 and 60 min. FE and FS will calculate using the following equation:

$$FE\ (\%) = (V_T/V_0)\ \ 100$$

$$FS\ (\%) = (V_t/V_T)\ \ 100$$

Where V_T is total volume after whipping, V_0 original volume before whipping, V_t is total volume after leaving at room temperature (26-28°C) for different times (0, 30, and 60 min).

Water-holding and fat binding capacity

Water holding and fat binding capacity were determined by the method of Balti *et al.* (2011). For water holding capacity, gelatin (0.5 g) was placed in a centrifuge tube and weight (tube with gelatin). Distilled water (50 mL) was added and held at room temperature for 1 h. The gelatin solutions then were centrifuged at 450 x g for 20 min. The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper after tilting to 45° angle. Water-holding capacity was calculated using the following equation:

$$WHC\ (\%) = \frac{\text{weight of the contents of the tube after draining}}{\text{weight of the dried gelatin}} \times 100$$

While for fat binding capacity, gelatin (0.5 g) was placed in a centrifuge tube and weight (tube with gelatin). Ten milliliter of soybean oil was added and held at room temperature for 1 h. The gelatin solution was mixed with vortex mixer for 5 sec every 15 min and then was centrifuged at 450 x g for 20 min. The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper after tilting to 45° angle.

Application of gelatin

Guava juice preparation

Guava was obtained from Makro Supercentre (Chiangrai, Thailand) and it was processed by following Wiley and Sons (2008). For juice extraction, ripe fruits were cut into small pieces followed by addition of water 250 mL/kg. The mix was cooked and stirred constantly, strained into cheese layer cloth and the juice was collected.

Fruit juice clarification

Gelatin was used as clarifying agent in guava juice for obtaining a clear juice by removing the suspended particles according to the method presented by Benitez and Lozano (2007) with slight modification. Fifty mL of Guava juice was added into 6 different tubes with an increasing gelatin concentration of 0, 0.16, 0.5, 1 and 2% (w/v). Gelatin was added into the guava juice and let to be rest for 1 min and let settle for 30 min. After the addition of each

gelatin concentration, tubes were agitated for few seconds. Once flocculation and sedimentation was completed, supernatant was carefully siphoned from every tube, and filter through filter paper and turbidity was determined using turbid meter (HI 93703, Hanna Instrument).

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).

RESULTS AND DISCUSSION

Yield

The yield of extracted gelatin which obtained from papain assisted extraction was 2.94% based on wet weight basis and 18.4% based on dry weight basis. Rahman and Jamalulail (2012) reported that the yield of extracted gelatin from chicken by products (bones and cartilages) with alkali pre-treatment and extracted at 60°C for 5 hours was 4.1% based on wet weight basis and 18% based on dry weight. 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).

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

Composition (%)	Gelatin	
	PAE	CBG
Moisture	7.56 ± 0.12[b]	11.75 ± 0.12[a]
Protein	40.06 ± 0.17[b]	91.97 ± 0.79[a]
Fat	1.13 ± 0.84[b]	5.09 ± 1.00[a]
Ash	1.47 ± 1.00[a]	0.84 ± 0.42[b]

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

Proximate Composition

The proximate compositions of gelatin extracted from chicken feet by using papain assisted extraction are shown in Table 1. Moisture content of gelatin obtained by papain assisted extraction was 7.56%. In comparison commercial bovine gelatin have moisture content 11.75%. 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 higher protein content with 91.97% compared to gelatin from papain assisted extraction with 40.06%. Commercial gelatin also has a higher percentage of fat content rather than extracted gelatin with 5.09% and 1.13%, respectively. The ash content of gelatin varies depends on the raw material and the method of processing (GMIA, 2012). The ash content in gelatin from papain assisted extraction was significantly different with commercial bovine gelatin, with the ash content 1.47% and 0.84%, respectively. According to Wasswa *et al*. (2007) the ash content in gelatin powder should not exceed 2%.

Color

The color of gelatin obtained from papain assisted extraction in comparison with commercial gelatin is shown in Table 2. Extracted gelatin showed different attributes of color *L**, *a** and *b** (*P < 0.05*), where the big difference was observed between the extracted gelatin and the commercial gelatin. The higher in lightness value was found in extracted gelatin with 80.16 ± 0.07, while commercial bovine gelatin with 69.70 ± 0.04. The color of gelatin are depends on the raw materials and the extraction method. However this attribute does not influence the properties of gelatin or reduce its function. According to Rahman and Jamalulail, (2012), chicken feet gelatin has value of *L**, *a** and *b** significantly higher than the commercial bovine gelatin with *L** values 42.94 ± 0.69 for chicken feet gelatin and 34.40 ± 0.03 for commercial bovine gelatin. While *a** value for chicken feet gelatin is 2.82 ± 0.23 and 1.87 ± 0.12 for commercial bovine gelatin. For value of *b**, chicken feet gelatin shows the reading 11.42 ± 0.20 and commercial gelatin at -4.68 ± 0.08.

Table 2 Physico-chemical properties of chicken feet gelatin and commercial bovine gelatin

Properties	Gelatin	
	PAE	CBG
Color attributes		
*L**	80.16 ± 0.07[a]	69.70 ± 0.04[b]
*a**	0.85 ± 0.01[b]	2.36 ± 0.10[a]
*b**	12.09 ± 0.01[a]	12.09 ± 0.01[a]
pH	5.65 ± 0.06[a]	5.03 ± 0.03[b]
Viscosity (cP)	2.98 ± 0.17[b]	5.29 ± 0.73[a]
Setting point (°C)	20.00 ± 0.01[a]	22.00 ± 0.39[a]
Melting point (°C)	30 ± 1.00[a]	30 ± 1.00[a]
Foaming properties (%)		
FE	1.10E2 ± 0.00[a]	1.12E2 ± 0.87[a]
FS 30 min	95.45 ± 0.00[a]	79.16 ± 0.00[b]
FS 60 min	90.90 ± 0.00[a]	79.16 ± 0.00[b]
Foaming properties (%)		
EAI (m^2/g)	10.93 ± 0.79[b]	16.50 ± 0.03[ab]
ESI (min)	71.30 ± 0.00[ab]	35.96 ± 0.87[b]

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

pH and viscosity

The pH value of the extracted gelatin was higher than commercial bovine gelatin with the respective values 5.65 and 5.03. The pH value of gelatin is influenced by the type and strength of the chemical that used during extraction procedure (Songchotikunpan *et al*., 2008). Previous studies on the pH values of chicken feet gelatin showed higher value (6.15) when compared with the commercial bovine gelatin (5.57) (Rahman and Jamalulail, 2012). On the contrary, 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).

Viscocity is the second most important commercial physical property of gelatin. The viscosity values of gelatin solution (6.67% concentration) obtained from extracted and commercial gelatin were 2.98 and 5.29cP. The differences of viscosity value were affected by molecular size, pH and molecular distribution. Rahman and Jamalulail, (2012) reported that the viscosity of chicken feet (bones and cartilages) gelatin from alkali pretreatment were lower compared to CBG in their percentage at 4.96% and 6.32%.

Fourier transform infrared spectroscopy

FTIR spectroscopy has been used to monitor the functional group and secondary structure of the gelatin (Kaewruang *et al*., 2013). Figure 1 shows the FTIR spectra of the extracted gelatin from chicken feet and commercial bovine gelatin. FTIR spectrum of the extracted gelatin is shown the major peaks in the amide region. Chicken feet gelatin showed the vibration peak at the wave numbers 1,745.62 cm^{-1} to amide I, 1,553.19 cm^{-1} to amide II, 1,238.51 cm^{-1} to amide III, 2,926.42 cm^{-1} to amide B, and 3,322.84 cm^{-1} to amide A. 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).

Electrophoresis analysis

Protein pattern of papain assisted extraction gelatin and commercial gelatin are shown in Figure 2. The papain assisted extraction gelatin shows the major protein band with the molecular weight of 130 kDa and 143 kDa. However, the commercial bovine gelatin has no identified major protein bands exhibited. The low molecular weight of the extracted gelatin with presence of papain was due to partial inactivation of the protease (Nalinanon *et al*., 2008).

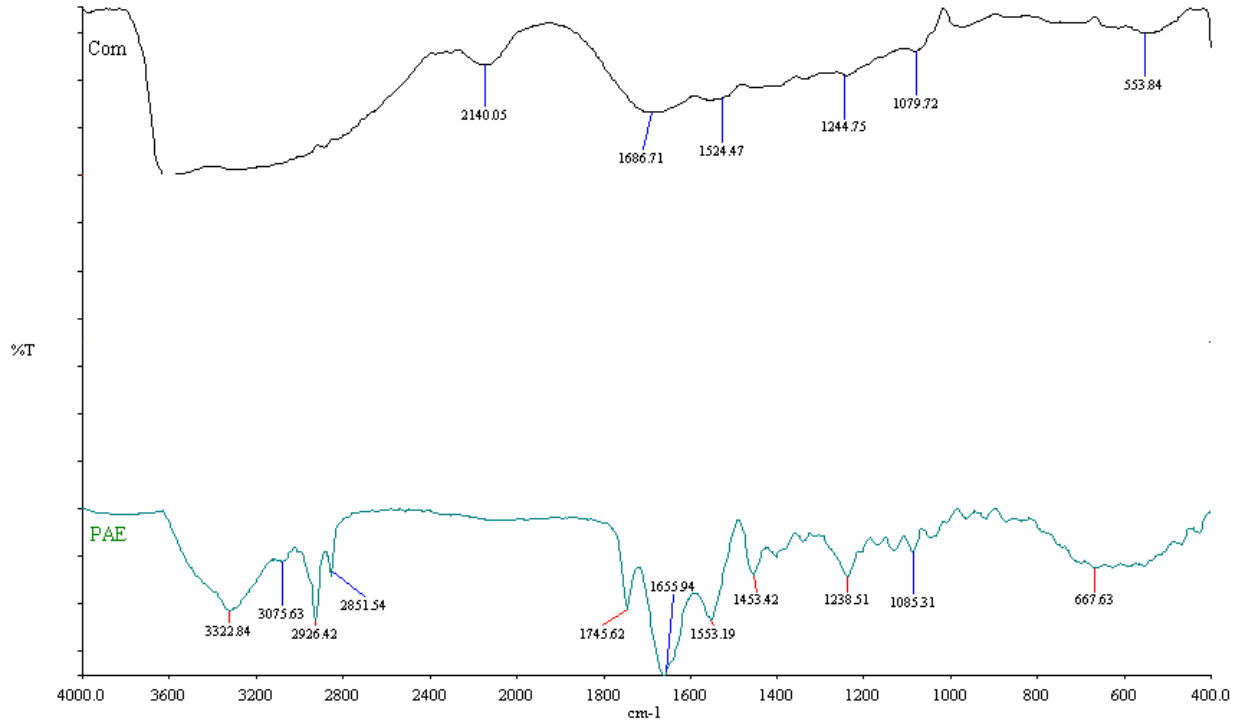

Figure 1 FTIR spectra of gelatin from chicken feet (PAE) and commercial bovine gelatin (Com= CBG) with spectra range at 400-4000 cm^{-1} was ratio and 32 scans at a resolution of 4 cm^{-1}

The FTIR spectra of commercial bovine gelatin amide I, II, and A were noticeable at 1,686.71 cm^{-1}, 1,524.47 cm^{-1}, and 3,619.04, respectively. Sinthusamran *et al*., (2014) reported that amide I band of gelatin extracted from skin of seabass was almost similar which appeared at 1643-1645 cm^{-1}. 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. According to Tu *et al*., (2013), similar result for amide II for gelatin from bighead carp scales by ultrasound assisted extraction where the band exhibited between 1,539.42 cm^{-1} to 1,541.67 cm^{-1}. 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).

Setting and melting point

The setting and melting points of papain assisted extraction gelatin are shown in Table 2. Papain assisted extraction gelatin and commercial bovine gelatin have setting point at 20 and 22°C, respectively. Furthermore, melting point of extracted gelatin was not significantly

different when compared with commercial bovine gelatin (30°C). Tavakolipour (2011) found that fitofague's acidic gelatin has setting point 20°C and mamalian acidic and alkaline gelatine have setting point range 27 to 32°C. Furthermore, Ninan *et al*., (2010) reported that higher setting and melting points can expand the range of gelatin application. They also found that carp had setting point 21.1°C and mammalian gelatin had setting point at range 23.7 to 24.2°C. Mohtar *et al.*, (2010) mentioned that increase gel strength of gelatin gel is accompanied by increase of melting point. While Rahman and Jamalulail (2012) found that the melting point of chicken feet (bones and cartilages) gelatin was 26.7°C. According to Karim and Bhat (2009) porcine and bovine gelatins, the melting points range from 20 to 25°C and 28 to 31°C.

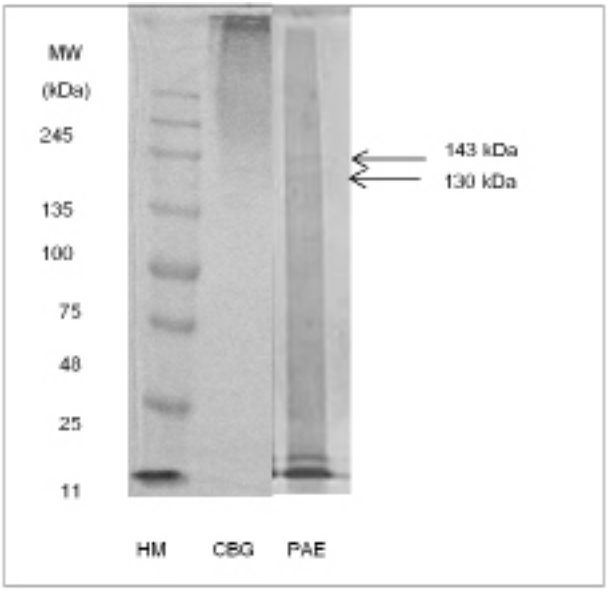

Figure 2 SDS-PAGE of gelatin from chicken feet. HM: MW markers, CBG: commercial bovine gelatin, and PAE: Papain assisted extraction gelatin with a 7.5% (*v/v*) running gel and 4% (*v/v*) stacking gel.

Emulsifying and foaming properties

Emulsifying properties of commercial bovine gelatin and extracted gelatin at the same concentration are shown in Table 2. At the same concentration used, EAI of extracted gelatin was not significantly different with CBG as well as with the value of ESI in comparison with commercial bovine gelatin (*P>0.05*). Gelatin act as emulsifier and use as water-in-oil emulsion such as low fat margarine, butter, salad dressing and whipped cream is due to the amphoteric property with the hydrophobic area on peptide chain. Furthermore, emulsifier can be used in food manufacture to improve physicochemical properties of food, to improve stability and to give the ability of food formation (Koli *et al*., 2012).

The foam expansion (FE) of extracted gelatin and CBG, litteraly shown not signifant different value (*P>0.05*). While for foam stability of extracted gelatin were slightly higher than CBG (*P>0.05*), where papain assisted extraction has foam stability (FS) with 95.45% and CBG has FS with 79.16%. This result indicated that extracted gelatin might form film with strong and great elasticity and leading to stable foam (Jellouli *et al*., 2011). Foaming properties might be influenced by the source of protein content, composition and intrinsc of protein, as well as conformation of protein in solution and in air/water interface (Balti *et al*., 2011). Foam capacity can improved by exposing the hydrophobic residue, while foam stability can increased by protein concentration. By adding gelatin to foamed desserts like yogurt, ice creams and mousses, gelatin can depresses the surface tension of water, resulting the formation of foam by mechanical whipping or injection of gas (Haug and Draget, 2011).

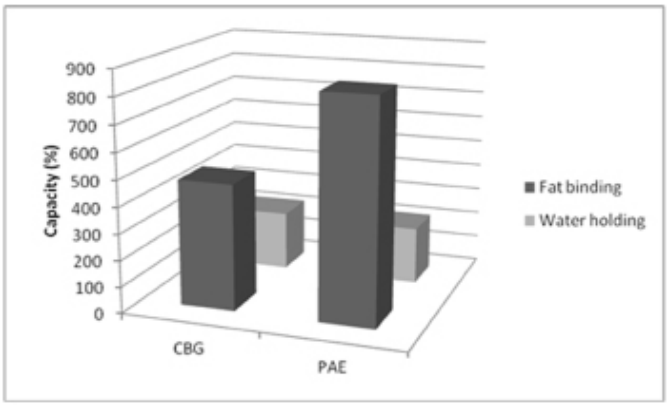

Figure 3 Water holding capacity and fat binding capacity of gelatin from chicken feet (PAE) and commercial bovine gelatin (CBG).

Water holding and fat binding capacity

Water holding capacity (WHC) and fat binding capacity (FBC) are functional properties that influenced to the texture profile by the interaction between component such as water, oil, and other components. The commercial gelatin has WHC slightly higher than extracted gelatin (Figure 3). The high value of WHC indicates the existance of great numbers of pores and voids within the structures of gelatin (Montoya *et al*., 2011). The functional properties in food system is depend on WHC which refers to the ability of protein to retain water against it gravitational force within protein matrix (Koli *et al*., 2012). The WHC of gelatin have functions for reducing drip loss and impairing juiciness in meat or frozen fish products when thawed or cooked. WHC can also secure good texture and taste of the meat products.

FBC of extracted gelatin and CBG is also shown in Figure 3. The extracted gelatin has FBC higher than that of the CBG. Fat binding capacity widely used in low-as fat replacer, low-carb as binding agent and low-calorie as fat replacer and binding agent in food products. Fat binding capacity is depends on the degree of exposure of the hydrophobic residues inside the gelatin (Jellouli *et al*., 2011).

Fruit juice clarification

To obtain a clear juice the suspended particles that exhibited in juice have to be removed which is known as clarification or fining process. Clarification is common method to use in common industrial practice. This clarifier agent works by sticking to the particles or by using charged ions that cause particles to stick to each other, which make them heavy and sink to the bottom by the action of gravity force resulting clear juice (Benitez and Lozano, 2007). Fining reaction is primarily influenced by pH where the positively charged gelatin molecule neutralize the negatively charged colloidal particel.

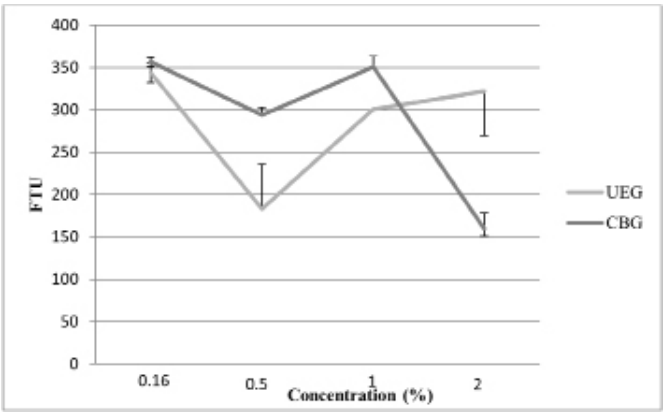

Figure 4 Effect of gelatins from chicken feet by papain assisted extraction (PAE) and commercial bovine gelatin (CBG) as clarifier at different concentrations.

Figure 4 shows the effect of papain assisted extraction gelatin at different concetrations of addition compare with the commercial bovine gelatin. Turbidity of guava juice with addition of gelatin at different concentrations was showed different results, where the highest value of turbidity are exhibited at 1% concentration for papain assisted extraction and commercial bovine gelatin with 484 and 371 FTU, respectively. Furthermore, the lowest turbidity value are purchased at 0.16% from extracted gelatin with 204.5 FTU and 2% from CBG with 181.5 FTU. The lowest value of turbidity was needed, because its contributed to the reduction of active gelatin sites resulting clear juice. The increase value in turbidity at 1% concentration was due to the non-flocculated particles which still remaining in suspension (Benitez and Lozano, 2007).

CONCLUSION

Gelatin from chicken feet was successfully extracted by using papain assisted extraction method. However, the yield is relatively low and the properties of extracted gelatin slightly different compare with commercial bovine gelatin. For instance, the foam expansion and foam stability of extracted gelatin were greater than commercial bovine gelatin. On the contrary the viscosity value of commercial bovine gelatin was higher compare to papain assisted extraction gelatin. However, as the overall results from physicochemical properties and application of extracted gelatin as guava juice clarifier, this study indicated that gelatin from chicken feet can be proposed as an alternative gelatin.

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REFERENCES

Ahmad, M. and Benjakul, S. 2011. Characteristics of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as influenced by acid pretreatment and extraction time. Food Hydrocolloids 25(3): 381-8.

Almeida, F., Lannes, S., Calarge, A., Farias, B. and Santana, C. 2012. FTIR Characterization of gelatin from chicken feet. Journal of Chemistry and Chemical Engineering 1029-1032.

AOAC. 2000. Official methods of analysis (17th ed). Washington, DC: Association of Official Analytical chemist.

Balti, R., Jridi, M., Sila, A., Souissi, N., Nedjar-Arroume, N., Guillochon, D. and Nasri, M. 2011. Extraction and functional properties of gelatin from the skin of cuttlefish (*Sepia officinalis*) using smooth hound crude acid protease-aided process. Food Hydrocolloids 25(5): 943-50.

Baziwane, D. and He, Q. 2003. Gelatin: The paramount food additive. Food Reviews International 19(4): 423-35.

Benitez, I, E. and Lozano, E, J. 2007. Effect of gelatin on apple juice turbidity. Latin America Applied Research 37: 261-266.

Cheow, CS., Norizah, MS., Kyaw, ZY. and Howell, NK. 2007. Preparation and characterisation of gelatins from the skins of sin croaker (*Johnius dussumieri*) and shortfin scad (*Decapterus macrosoma*). Food Chemistry 101(1): 386-391.

Duan, R., Zhang, J., Xing, F., Konno, K. and Xu, B. 2011. Study on the properties of gelatins from skin of carp (*Cyprinus carpio*) caught in winter and summer season. Food Hydrocolloids 25(3): 368-73.

Haug, I, J. and Draget, K, I. 2011. Gelatin. In G,O, Philips. and P, A, Williams. (Eds). Handbook of food proteins, p. 92-115. UK: Elsevier.

Jallouli, K., Balti, R., Bougatef, A., Hmidet, N., Barkia, A. and Nasri, M. 2011. Chemical composition and characteristics of skin gelatin from grey triggerfish (*Balistes capriscus*). Food Science and Technology 44 (9): 1965-1970.

Kaewruang, P., Benjakul, S., Prodpran, T. and Nalinanon, S. 2013. Physicochemical and functional properties of gelatin from the skin of unicorn leatherjacket (*Aluterus monoceros*) as affected by extraction conditions. Food Bioscience 2: 1-9.

Karim, AA. and Bhat, R. 2008. Gelatin alternatives for the food industry: recent developments, challenges and prospects. Trends in Food Science & Technology 19(12): 644-56.

Karim, AA. and Bhat, R. 2009. Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids 23(3): 563-76.

Karnjanapratum, S. and Benjakul, S. 2014. Glycyl endopeptidase from papaya latex: partial purification and use for production of fish gelatin hydrolysate. Food Chemistry 165: 403-411.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W. and Shahidi, F. 2010. Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. Food Hydrocolloids 24: 164-171.

Koli, J., Basu, S., Nayak, B., Patange, S., Pagarkar, A. and Gudipati, V. 2012. Functional characteristics of gelatin extracted from skin and bone of tiger-toothed croaker (*Otolothes ruber*) and pink perch (*Nemipterus japonicas*). Food and Bioproducts Processing 90(3): 555-562.

Lasekan, A., Bakar, A, F. and Hashim, D. 2013. Potential of chicken by-products as sources of useful biological resources. Waste Management 33: 552-565.

Montoya, H, M., Ortega, S, H., Moroyoqui, C, J, F., Sandez, R, O., Jatomea, P, M., and Brauer, E, M, J. 2011. Giant squid skin gelatin: Chemical composition and biophysical characterization. Food Research International 44: 3243-3249.

Nalinanon, S., Benjakul, S., Visessanguan, W., and Kishimura, H. 2008. Improvement of gelatin extraction from bigeye snapper skin using pepsin-aided process in combination with protease inhibitor. Food Hydrocolloids 22: 615-622.

Ninan, G., Joseph, J. and Abubacker Z. 2010. Physical, mechanical, and barrier properties of carp and mammalian skin gelatin films. Journal of Food Science 75(9): E620-E6.

Niu, L., Zhou, X., Yuan, C., Bai, Y., Lai, K., Yang, F. and Huang, Y. 2013. Characterization of tilapia (*Oreochromis niloticus*) skin gelatin extracted with alkaline and different acid pretreatments. Food Hydrocolloids 33(2): 336-41.

Rahman, N. and Jamalulail, S. 2012. Extraction, physicochemical characterizations and sensory quality of chicken feet gelatin. Borneo Science 30: 1-13.

Rawdkuen, S., Chaiwut, P., Pintathong, P. and Benjakul. S. 2010. Three-phase partitioning of protease from *Calotropis procera* latex. Biochemical Engineering Journal 50(3): 145-149.

Saiut, S., Jongjareonrak, A. and Rawdkuen, S. 2012. Re-extraction, recovery and characteristics of skin gelatin from farmed giant catfish. Food Bioprocess Technology 5: 1197-1205.

Sarbon, Mhd N., Badii, F. and Howell, NK. 2013. Preparation and characterisation of chicken skin gelatin as an alternative to mammalian gelatin. Food Hydrocolloids 30(1): 143-51.

Sinthusamran, S., Benjakul, S. and Kishimura, H. 2013. Comparative study on molecular characteristics of acid soluble collagens from skin and swim bladder of seabass (*lates calcarifer*). Food Chemistry 138(4): 2345-2441.

Songchotikunpan, P., Tattiyakul, J. and Supaphol, P. 2008. Extraction and electrospinning of gelatin from fish skin. International Journal of Biological Macromolecules 42(3): 247-55.

Taheri, A., Abedian Kenari, AM., Gildberg, A. and Behnam, S. 2009. Extraction and physicochemical characterization of greater lizardfish (*Saurida tumbil*) skin and bone gelatin. Journal of Food Science 74(3): E160-E5.

Tavakolipour, H. 2011. Extraction and evaluation of gelatin from silver carp waste. World Journal of Fish and Marine Sciences 3(1): 10-15.

Tu, Z., Huang, T., Wang, H., Sha, X., Shi, Y., Huang, X., Man, Z. and Li, D. 2013. Physico-chemical properties of gelatin from bighead carp (*Hypophthalmichthys nobilis*) scales by ultrasound-assisted extraction. Journal Food Sciences. 1-9.

Wasswa, J., Tang, J. and Gu, X. 2007. Utilization of fish processing by-products in the gelatin industry. Food Reviews International 23(2): 159-74.

Wiley, J. and Sons. 2008. Guava. In Y, H, Hui. (Ed). Handbook of fruits and fruit processing, p. 598-606. West Sacramento, CA.