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ORIGINAL PAPER

Effects of acid type extraction on characterization and sensory profile of duck feet gelatin: towards finding bovine gelatin alternative

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Abstract This study was conducted to characterize the physicochemical properties of duck feet gelatin extracted by using different acid solutions which is hydrochloric acid (DFCl), acetic acid (DFAa), lactic acid (DFLa), and citric acid (DFCa). Proximate analysis, bloom strength, amino acid composition and sensory profiles were evaluated. Duck feet gelatin had higher bloom strength with 225.53, 334.17, 322.17 and 322.63 g for DFCl, DFAa, DFLa and DFCa, respectively, compared to commercial bovine gelatin with 216.63 g. DFCa had the highest imino acid with value of 23.01% compared to other gelatin sample. Sensory profiles in powder and gels form of gelatin were evaluated by 12 trained panellists using quantitative descriptive analysis (QDA). Overall, gelatin powder of DFCl had the highest degree of acceptability with average score of 11.15, but there's no significant different between DFCa. While for gelatin gel, DFCa had the highest intensity of fracturability, firmness and the overall degree of acceptability score is 10.48, but there is no significant difference among other acid treatments. It was found that all extracted duck feet gelatin has high potential for application as an alternative to commercial gelatin that already available in market.

Keywords Duck feet gelatin · Acid treatment · Amino acid · Gelatin alternative

Introduction

Gelatin comes from animal origin which is obtained from collagen protein through acid or alkaline hydrolysis. Gelatin is widely used in food industry as an additive for stabilizing, gelling and emulsifying. Gelatin also use in pharmaceutical, cosmetic and photography industry. Alternative sources of gelatin have gained an attention [1–4] because the main gelatin sources from mammalian (bovine and porcine) create a controversy due to religious factors and diseases. The alternatives to mammalian gelatin is gelatin from marine sources, but Nieuwenhuizen et al. [5] reported that 90% of allergic reaction comes from eight types of food which included fish and shellfish. Hence, the raw material from fish origin may not be suitable for certain people to consume because of the allergic factor [6].

According to Karim and Bhat [2], it was expected that poultry skin and bones will be one of the main gelatin sources in the near future. Poultry by-products including skin and feet contains large amounts of collagen. Several studies were done on poultry such as chicken skin [7–10], chicken feet [8, 11, 12], bird feet [13], silky fowl feet [14] and duck feet [3, 15, 16]. Huda et al. [17] reported that duck feet collagen can improve the physicochemical properties of sardine surimi.

According to Maurer [18], there are several ways to handle poultry offal (heads, feet and inedible viscera). Usually, all offal except the blood and condemned birds is floated in water from the processing areas to an accumulation area for

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removal by trucks. This waste can be minimize by utilize it as a source for gelatin production. Malaysia is one of the top main producers of duck meat and the average duck meat production around the world from year 1992 to 2012 shown that Asia region contribute to 80.5% of the total productions (FAO 2014). Hydrochloric acid pre-treatment for gelatin extraction is an old and common method that recently used for duck feet gelatin extraction [19, 20] but to our knowledge effects of different acid type for duck feet gelatin was not investigated. So the objective of the present study was to characterize the physicochemical properties and to evaluate the sensory profiles of gelatin extracted from duck feet using different acid solution and to compare it with commercial bovine gelatin.

Materials and methods

Materials

Samples of duck feet were purchased from Perak Duck Food Industries Sdn. Bhd, (Perak, Malaysia). It was transported to the laboratory in ice storage and it was stored at −20 °C prior to use. Commercial bovine gelatin (CBG) was purchased from Sigma Aldrich (St. Louis, MO, USA). All reagent and chemical used were analytical grade.

Gelatin extraction

Gelatin extraction was done following Kim et al. [8] with some modifications. The duck feet nails and superficial fat were discarded. Thawed duck feet were cut into small pieces. Then, it was grinded by using a 12 mm plate meat mechanical mincer (Model EVE/ALL-12, Rheninghaus, Torino, Italy). The grounded duck feet were washed with tap water (1:5 w/v) at room temperature (28 °C) for 10 min before being defatted by using 10% butanol w/v (1/20) for 12 h with continuous stirring. After defatting process, the sample was washed for about 5 min to remove any remaining butanol. Grounded duck feet was treated with four different acid solution which is 0.1 M hydrochloric, acetic, lactic and citric acid with ratio 1/10 (w/v) for 24 h at 7 °C by batch. Then, it was neutralized with flowing tap water prior to extraction process. Gelatin was extracted in distilled water with samples to ratio 1:2 (w/v) at 75 °C for 2 h in a steel tank. The solution was filtered by using Whatman filter paper No. 4 and it was freeze (−18 °C) before being lyophilized (Labconco Freezedry system, Kansas City, MO, USA). Obtained dry gelatin was grounded (Panasonic kitchen grinder) before being analyse. Gelatin extraction yield is calculated based on wet weigh of raw material.

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

Proximate composition of gelatin

The moisture, ash and fat content of extracted and dried gelatin were determined according to the AOAC (2000). The protein content was determined by Kjeldahl method (AOAC 2000) with a factor of 5.55 to convert the nitrogen value to gelatin protein.

Determination of bloom strength

Bloom strength of gelatin gel was determined according to Gelatin Manufacturers of Europe Monograph as described by Mhd Sarbon et al. [9] using a texture analyzer TA.XT Plus (Stable Micro Systems, Godalming, UK) with a 5 kg load cell. Gelatin solution of 6.67% was prepared in bloom jar by weighing 7.5 g of powdered gelatin and 105 mL distilled water. The solution was swirled and left it for 3 h at room temperature. Then, the solution was heated at 60 °C for 20 min to completely dissolve the gelatin powder. It was cooled for 15 min at room temperature before keeping in refrigerated water bath at 10 °C for 16–18 h for gel maturation. The bloom strength (g) of gel was measured with standard radius cylinder (P/0.5R) probe with depth of 4 mm at 0.5 mm/s.

Determination of amino acid composition

The amino acid composition of the samples was analysed according to Aronal et al. [21] by digesting the samples for 24 h at 110 °C in an oven with 5 mL of 6 N HCl in sealed glass tubes. An aliquot of the hydrolysate was taken and 0.4 mL AABA (alpha amino butyric acid) (50 µmol/mL) was added to it as the internal standard. Then 100 mL of distilled water was added to the aliquot. The aliquot was then filtered by using filter paper followed by a syringe filter (0.45 µm). All samples was derivative with an AQC (6-aminoquinolyl-*N*-hydroxysccinimidyl carbamate) reagent and borate buffer (200 mM) before being separated using high performance liquid chromatography (HPLC) using eluent A (AccQ Taq™ concentrate, Waters) and eluent B (Acetonitrile 60%, Sigma). HPLC with Waters brand system consisted of the following items: a multi-fluorescence detector Waters 2475 (excitation at 250 nm and emission at 395 nm), a Waters 717 auto-sampler and a Waters binary 1525 HPLC pump and bus satin model. The column used was AccQ-Tag™ size of 3.9 × 150 mm (Waters, Ireland). The eluent flowed at a rate of 1 mL/

min. Chromatographic peaks were integrated, identified and quantified with Breeze™ software version 3.20 by comparing it to known standards (Amino acid standard H; Pierce, Rockford, IL, USA).

Sensory evaluation

Quantitative Descriptive Analysis (QDA) sensory test involved twelve trained panellist (six men and six women, age ranges 23–47 years old) which were selected through pre-screening. Analysis was done according to Boran et al. [22] with modification. Training of the selected panellist took 10 h which included the terminology of product development (Table 1a and b), introduction to descriptive scaling scale (150 mm scale) and practices using references. Each member of the panel observed the differences in appearance, odour and texture of gelatin powder and gel.

Sample preparation of gelatin powder was done by filled half of the 15 mL plastic cup. While for gelatin gel, samples were prepared by dissolved 6.67% of gel powder in distilled water at 60 °C for 30 min. Then, 10 mL of samples were poured into 15 mL plastic cups. For elasticity, gelatin gel was pour into 200 mm × 6 mm straw, it was cooled at room temperature for about 10 min and placed it in chiller for 16–18 h. Then, the straw was cut and each panellist was given with 3 mm gelatin samples. All gelatin samples were coded with three digit random numbers and presented on a tray to panelists who were seated in individual booth in Sensory Laboratory Lab, Department of Food Science and Technology, School of Industrial Technology, USM (T = 25 °C and RH = 60%). Each panelist evaluated each of five samples; DFCl, DFAa, DFLa, DFCa and CBG for gelatin powder and gelatin gel on different days (six sessions). Sensory evaluation was conducted on duplicates for each samples.

Statistical analysis

Statistical research was done using SPSS software (SPSS 17.0 for Windows, SPSS Inc, Chicago IL, USA) by performing one way analysis of variance (ANOVA). Duncan tests ($P < 0.05$) was used to determine which samples were significantly different. While, the QDA data was converted to spider web by using Microsoft excel.

Results and discussions

Proximate composition and extraction yield of duck feet gelatin

The proximate analysis based on wet basis in Table 2 showed that protein content among different acid treated of duck feet was highest in DFLa with 89.67%. There is no significant different in protein content of DFAa and DFCa. It shows that protein content of DFAa is the lowest with 86.40% might be due to high moisture content which is 10.24%, but all acid treated was within the prescribed limit of moisture content which is less than 15% [23]. DFLa had the lowest moisture content with 6.29%. DFAa shows the lowest fat content with 0.57% compared to other acid treatment but slightly higher compared to commercial bovine gelatin with 0.10%. There were no significant different in fat content between acid treatments of DFCl and DFCa. DFLa also had the lowest ash content compared to the other acid treated with 0.58%. The extraction yield of different acid treated also presented in Table 2. Results show that there is no significant difference observed in extraction yield and the extraction yield is around 4% for acidic extraction methods. Similar results were reported by other researchers. Gelatin yield from duck feet gelatin is 4.09–5.75% [19], chicken feet is 5.33% [24],

Table 1 **a** Sensory vocabulary for analysis of gelatin powder and **b** gelatin gel

Sensory attribute	(0–15) Reference	Definition
(a)		
Lightness*	Dark–light	Light intensity of sample
Animalic odour**	None–strong	Open the lid slightly and sniff at one time (20 mm from cup)
Coarse of particle**	Fine particles–large particles	See the coarse particle and touch with index finger
Stickiness**	None–sticky	Stickiness on the skin between thumb and index finger
(b)		
Transparency*	Cloudy–clear	See the printed number at the bottom of the cup from the top of the sample
Animalic odour**	None–strong	Open the lid and sniff at one time (20 mm from the cup)
Fracturability**	Low–high	Force to fully rupture the sample using index finger
Firmness**	Soft–hard	Force require to partially compress the sample between thumb and index finger
Elasticity*	Low–high	Pull the sample till 50 mm

*0: least acceptable 15: most acceptable

**0: most acceptable 15: least acceptable

Table 2 Proximate analysis, extraction yield and bloom strength of gelatin

	Extraction yield	Moisture (%)	Protein (%)	Ash (%)	Fat (%)	Bloom strength (g)
DFCl	4.09 ± 0.12^{a}	10.13 ± 0.31^{c}	87.74 ± 0.52^{b}	1.35 ± 0.02^{bc}	0.75 ± 0.01^{c}	225.53 ± 6.50^{b}
DFAa	3.97 ± 0.08^{a}	11.19 ± 0.25^{d}	86.40 ± 0.35^{a}	1.08 ± 0.06^{b}	0.57 ± 0.09^{b}	334.17 ± 1.29^{d}
DFLa	4.26 ± 0.15^{b}	6.29 ± 0.08^{a}	89.67 ± 0.20^{c}	0.58 ± 0.02^{a}	1.20 ± 0.06^{d}	322.17 ± 3.60^{c}
DFCa	4.03 ± 0.04^{a}	10.24 ± 0.14^{c}	86.62 ± 0.31^{a}	1.25 ± 0.06^{b}	0.70 ± 0.06^{c}	322.63 ± 4.10^{c}
CBG	4.21 ± 0.11^{ab}	7.30 ± 0.14^{b}	89.48 ± 0.37^{c}	1.60 ± 0.37^{c}	0.10 ± 0.01^{a}	216.63 ± 4.54^{a}

DFCl duck feet treated with hydrochloric acid, *DFAa* duck feet treated with acetic acid, *DFLa* duck feet treated with lactic acid, *DFCa* duck feet treated with citric acid, *CBG* commercial bovine gelatin

[a,b,c]Values are mean of triplicate of each samples with ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$)

1.72–5.33% [25], and 5.97–7.835% for different parts of chicken feet tissue [26].

Gelatin can absorb or release moisture depending on the humidity of the surrounding air. Many factors can influenced the moisture content of gelatin such as drying time, humidity, storage room and type of packaging. Schrieber and Gareis [27] noted that if water content exceeds 16%, there is a risk of lump formation and microbiological growth. So, to avoid the water absorption, vapour tight packaging is recommended. From Table 2, results showed that, gelatin extraction process was done effectively as Rahman and Jamalulali [18] mention, under the Food Act (2011) Malaysia, the Food Act 1983 and Food Regulations 1985, the percentage of ash for gelatin powder should not exceed 3%. Also, Ledward et al. [28], stated that the ash content should be < 2%.

Bloom strength of duck feet gelatin

Bloom strength of gelatin are 334.17, 322.63, 322.17, 247.97 and 226.53, 216.63 g for DFAa, DFCa, DFLa, CFG, DFCl and CBG, respectively. There is significant different in bloom value of DFAa and it had the highest bloom value with 334 g. While for DFLa and DFCa, no significant different in bloom strength value but all acid treated are included in high quality of gelatin because it is more than 200 g bloom value.

Previous study by Mhd Sarbon et al. [9] showed that the bloom strength of chicken skin is higher with 355 g. The primary property of gelatin is its gelling effect [27] and the bloom strength is the most important physical properties of gelatin [2]. According to Cheow et al. [29], it is the key parameter in determining the quality of gelatin. Gelatin was grouped into high (220–300 g), medium (150–220 g) and low (< 150 g) [9]. Present study showed that all duck feet gelatin was included in high bloom strength. High bloom strength contribute to high melting temperature and generally gelatin with high bloom value also show higher viscosity because of higher proportion of cross-linked component of ß and α chain.

Amino acid content for duck feet gelatin

Table 3 shows the results of amino acid content for duck feet gelatin and commercial bovine gelatin. The results for average amino acid composition of all gelatin were similar. Glycine is the major amino acid in all gelatin [1, 30]. Glycine and proline content results for DFAa, DFLa and DFCa were in agreement with those found by Khiari et al. [30] for different types of treated acid which is acetic acid, lactic acid and citric acid. Tyrosine and histidine content are very low in all gelatin and the present study didn't detect the cysteine. According to Khiari et al. [30], detection of cysteine indicate that there were possible contamination by non-collagenous proteins during extraction process. Hence, the extraction process was done without contamination as cysteines were not detected. Previous study by Huda et al. [15] also showed that there is no cysteine detected as duck feet collagen included in type 1 collagen. For duck feet gelatin, DFCa had the highest imino acid (proline and hydroxyproline) compared to the other duck feet gelatin and commercial bovine gelatin with value of 22.55, 22.54, 22.81, 23.01 and 21.27% for DFCl, DFAa, DFLa, DFCa and CBG, respectively.

The other important properties of gelatin are the amino acid especially imino acids content. Imino acids are responsible for stability of triple helix structure through hydrogen bonding between free water molecules and hydroxyl group of hydroxyproline in gelatin [9]. Gelatin with high imino acid usually had higher bloom strength due to its stability of triple helix structure.

Sensory analysis of gelatin powder and gelatin gel

Figures 1 and 2 shows the spider web for QDA sensory analysis of gelatin powder and gelatin gel for DFCl, DFAa, DFLa, DFCa and CBG. From Fig. 1, panelists indicated that different sample were differ in terms of lightness, coarse, stickiness of particle and odour. Panelists indicated that gelatin powder of CBG had the lowest score of lightness compared to other gelatin powder. According to Sukkwai

Table 3 Amino acids composition of gelatin

Amino acids (%)	DFCl	DFAa	DFLa	DFCa	CBG
Alanine	10.75 ± 0.04^{b}	10.86 ± 0.06^{bc}	9.96 ± 0.00^{a}	10.26 ± 0.02^{b}	11.00 ± 0.11^{c}
Arginine	6.15 ± 0.04^{a}	6.17 ± 0.00^{a}	6.48 ± 0.01^{b}	6.73 ± 0.02^{c}	6.86 ± 0.08^{d}
Aspartic acid	4.12 ± 0.01^{c}	4.24 ± 0.01^{c}	3.67 ± 0.00^{a}	3.94 ± 0.01^{b}	3.91 ± 0.03^{b}
Cysteine	ND	ND	ND	ND	ND
Glutamic acid	7.47 ± 0.02^{d}	7.45 ± 0.04^{d}	6.68 ± 0.06^{b}	6.79 ± 0.04^{c}	6.59 ± 0.01^{a}
Glycine	29.03 ± 0.01^{b}	29.04 ± 0.05^{b}	30.77 ± 0.01^{d}	29.74 ± 0.08^{c}	28.12 ± 0.16^{a}
Histidine	0.75 ± 0.01^{a}	0.76 ± 0.00^{a}	0.86 ± 0.01^{b}	0.84 ± 0.01^{b}	1.00 ± 0.01^{c}
Hydroxyproline	10.28 ± 0.01^{b}	10.31 ± 0.01^{b}	11.01 ± 0.06^{c}	11.13 ± 0.09^{c}	9.46 ± 0.04^{a}
Isoleucine	1.44 ± 0.01^{d}	1.44 ± 0.01^{d}	1.36 ± 0.00^{b}	1.40 ± 0.02^{c}	1.23 ± 0.01^{a}
Leucine	3.17 ± 0.01^{d}	3.08 ± 0.04^{c}	2.97 ± 0.01^{a}	3.01 ± 0.03^{ab}	2.96 ± 0.01^{a}
Lysine	2.71 ± 0.01^{d}	2.74 ± 0.02^{d}	2.30 ± 0.02^{a}	2.51 ± 0.01^{b}	2.66 ± 0.00^{c}
Methionine	1.05 ± 0.01^{b}	1.07 ± 0.01^{b}	1.12 ± 0.01^{c}	0.76 ± 0.02^{a}	1.13 ± 0.00^{c}
Phenyalanine	2.03 ± 0.00^{a}	2.00 ± 0.01^{a}	2.16 ± 0.00^{b}	2.13 ± 0.06^{b}	2.05 ± 0.00^{a}
Proline	12.27 ± 0.00^{b}	12.23 ± 0.04^{b}	11.80 ± 0.01^{a}	11.88 ± 0.06^{a}	11.81 ± 0.06^{a}
Serine	3.57 ± 0.00^{bc}	3.47 ± 0.01^{a}	3.60 ± 0.01^{c}	3.52 ± 0.03^{ab}	5.01 ± 0.04^{d}
Threonine	2.42 ± 0.01^{a}	2.40 ± 0.06^{a}	2.57 ± 0.00^{b}	2.64 ± 0.02^{b}	3.41 ± 0.02^{c}
Tyrosine	0.60 ± 0.00^{b}	0.62 ± 0.01^{bc}	0.63 ± 0.01^{c}	0.60 ± 0.01^{b}	0.45 ± 0.00^{a}
Valine	2.24 ± 0.01^{c}	2.24 ± 0.00^{c}	2.11 ± 0.00^{a}	2.17 ± 0.00^{b}	2.39 ± 0.01^{d}
Imino acid (Hyp + Pro)	22.55 ± 0.01^{b}	22.54 ± 0.05^{b}	22.81 ± 0.06^{c}	23.01 ± 0.15^{c}	21.27 ± 0.02^{a}

DFCl duck feet treated with hydrochloric acid, *DFAa* duck feet treated with acetic acid, *DFLa* duck feet treated with lactic acid, *DFCa* duck feet treated with citric acid, *CBG* commercial bovine gelatin, *ND* not detected

Means with different lowercase letters within column of gelatin samples are significantly difference at $P < 0.05$

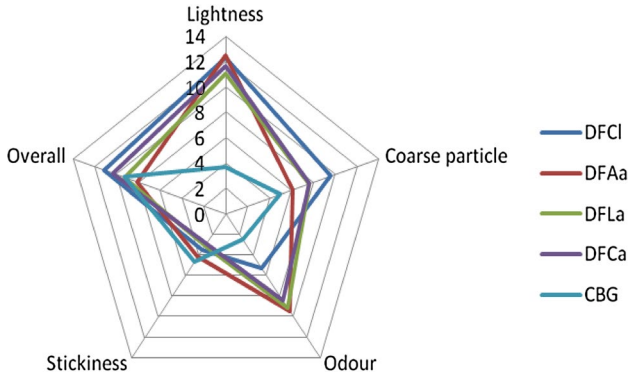

Fig. 1 Spider web for QDA sensory analysis of gelatin powder. *DFCl* duck feet treated with hydrochloric acid, *DFAa* duck feet treated with acetic acid, *DFLa* duck feet treated with lactic acid, *DFCa* duck feet treated with citric acid, *CBG* commercial bovine gelatin

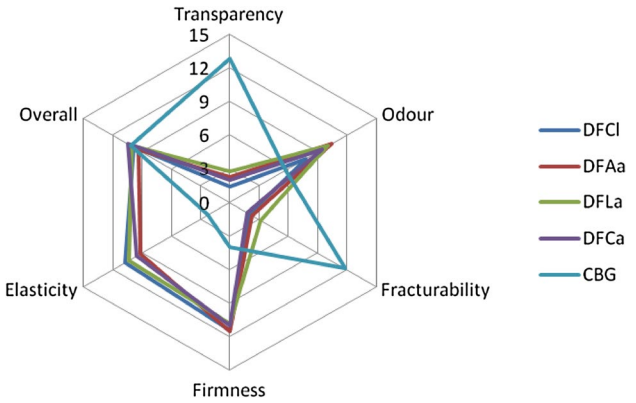

Fig. 2 Spider web for QDA sensory analysis of gelatin gel. *DFCl* duck feet treated with hydrochloric acid, *DFAa* duck feet treated with acetic acid, *DFLa* duck feet treated with lactic acid, *DFCa* duck feet treated with citric acid, *CBG* commercial bovine gelatin

et al. [31] differences in colour might be due to the different extraction conditions. The coarse of particle for gelatin powder of DFCa and DFLa were not significantly different from each other but they were significantly different from all other samples. In terms of coarse of particle, gelatin powder of DFCl were larger than gelatin powder of DFCa, DFLa, DFAa and CBG. Gelatin powder of DFCa and DFLa did not differ ($p > 0.05$) in size. Smaller particles can easily disperse in water than large particles. Odour of samples DFCl and CBG were significantly different from each other and all other samples. Whilst, DFAa, DFLa, and DFCa did not differ ($p > 0.05$) in their odour. Strong animalic odour in gelatin extracted from duck feet because of it high fat content. It was noted that odour and meaty flavour of duck meat are

stronger [32]. Hence, CBG had less odour due to lower fat content with 0.10% (Table 3). Stickiness plays an important role for gel formation of the gelatin. DFCa had the least stickiness and did not differ ($p > 0.05$) with DFCl and DFLa. Overall acceptance for gelatin powder indicates that, DFCl was the most acceptable and DFAa was the least acceptable. DFCl had the highest degree of acceptability with average score 11.15 but there were no significant different in overall acceptance of gelatin powder between DFCl and DFCa; CBG and DFLa.

Results as shown in Fig. 2 indicate that CBG in gels form were significantly different from gelatin of duck feet samples. DFCl, DFLa, DFCa were not significantly different. CBG was more transparent compared to extracted duck feet gelatin. The extracted duck feet gelatin less transparent compared to commercial gelatin because it is more turbid due to insoluble or foreign matter in the form of emulsions or dispersions which have become stabilized due to the protective colloidal action of gelatin (GMIA, 2012). CBG gel had less odour compared to DFAa, DFLa, DFCa and DFCl. These results were tally with gelatin powder. This is also because of different raw material of gelatin itself. CBG gels were more fracture and least firm compared to DFCl, DFAa, DFLa and DFCa. No significant ($p > 0.05$) different in DFLa and DFAa; DFAa, DFCl and DFCa in terms of fracturability. There is no significant different for firmness of DFAa, DFCl, DFCa and DFLa of gelatin gel. For elasticity, all extracted duck feet gelatin were more elastic compared to CBG. This three attributes (fracturability, firmness and elasticity) are related to the bloom strength of gelatin gel. Overall acceptance of gelatin gel show that, DFCa was the most acceptable with score 10.48 but there was no significant different among extracted duck feet gelatin of DFCa, DFLa, DFCl and CBG.

Conclusion

Sensory evaluation revealed that panellist tends to choose duck feet gelatin compared to commercial bovine gelatin and DFCa is more preferable due to high degree of overall acceptability in sensory evaluation. Besides that, DFCa gave high yield and high imino acid content but other acid treated also gave better properties as compared to commercial bovine gelatin. Present study indicate that duck feet gelatin has high quality than commercial bovine gelatin. Thus, duck feet can be a new potential for the alternatives raw material of gelatin and at the same time it can minimize the industrial by-product from poultry. Gelatin from duck feet also can act as an alternative to halal gelatin in the market for food products, pharmaceutical and nutraceutical.

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