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Physicochemical Properties of Duck Feet Gelatin Powder Extracted with Acetic Acid

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Abstract*— Physiochemical properties such as colour, amino acid composition, melting temperature and hydroxyproline content determines the quality of gelatin. In this study, 4% of acetic acid was used to produce gelatin powder from Pekin, Muscovy and Khaki Campbell duck feet. The duck feet gelatin powders were analysed for their physiochemical properties and compared with commercial bovine gelatin. For colour analysis, Pekin duck feet gelatin (PDFG) had the highest L* value (21.41), followed by Khaki Campbell duck feet gelatin (KCDFG, 20.57) and Muscovy duck feet gelatin (MDFG, 17.85). KCDFG and PDFG had the same a* values (redness) which was -0.05, while the a* value of MDFG was -1.49. Lastly, the b* values of duck feet gelatin powder were 6.17, 8.47 and 9.47 for PDFG, MDFG and KCDFG, respectively. Duck feet gelatin powder had a melting temperature value of 61.91°C, 48.62°C and 44.81°C for KCDFG, MDFG and PDFG, accordingly. PDFG had the highest hydroxyproline content, which was followed by KCDEG and MDFG with the values of 9.15, 8.00 and 7.63 g/100g, respectively. The main amino acids present in the duck feet gelatin powder were glycine (21.73%, 13.62%, 13.46% and 15.33%), proline (13.08%, 8.71%, 8.39% and 9.34%), hydroxyproline (12.57%, 8.05%, 7.84% and 9.39%) and alanine (8.79%, 5.77%, 6.03%, 6.65%) for CBG, KCDFG, MDFG and PDFG, respectively. Furthermore, the lowest amino acids observed were tyrosine, histidine, methionine and isoleucine. No trace of cysteine was observed. Pekin duck feet produced the best quality gelatin compared to Khaki Campbell and Muscovy duck feet.

***Keywords*— Gelatin; duck feet; physicochemical properties; amino acid composition.**

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I. INTRODUCTION

Gelatin, a high molecular weight fibrous protein is obtained from collagen by the process of thermal hydrolysis [1, 2]. It has a total protein of about 25 to 35% [1, 2]. Gelatin is produce by breaking the cross linkage between the peptide chains together with decomposition of polypeptide bonds [3, 4]. The helixes in the collagen are partly transformed and become distinguished from collagen [5]. Since gelatin consists of vast amounts of denatured collagen, it is similar to collagen molecule in amino acid composition. Nevertheless, the composition of amino acids in gelatin is not certainly characterized [6].

In Asia, chicken meat is the most consumed meat, followed by duck meat [7, 8]. Globally, about 84.2% of all duck meat are produced in Asia [9]. Duck meat also has the potential for use in the production of surimi. [10, 11]. The large scale production of ducks results in the production of large scale duck by-products such as feathers, viscera, bones and feet. Duck feet has been a popular raw material used in collagen and gelatin production [12, 13]. Duck feet collagen and gelatin have complex bones and tendons [14] and low gelling temperature which is a possible characteristics that can be used in micro-encapsulation of bioactive components [15].

The objective of this study was to produce gelatin powder using 4% acetic acid from Khaki Campbell, Muscovy and Pekin duck feet. The physicochemical properties of the gelatin powder such as colour, melting temperature, hydroxyproline content and amino acid profile were studied and compared with commercial bovine gelatin.

II. Materials and Methods

A. Materials

Both Muscovy and Khaki Campbell duck feet were bought from Kelantan, Malaysia. The Pekin duck feet were purchased from Duck Food Industries Sdn Bhd in Perak Malaysia. The commercial bovine feet were purchased from Halahel Sdn Bhd in Kedah, Malaysia. The duck feet were transported in an ice box fill with ice to the lab for further analysis. Prior to the anlysis, the duck feet were stored at -18°C. The reagents and chemicals used were of analytical grade.

B. Extraction of Gelatin

Duck feet gelatin was extracted using a modified method of Kuan et al. [16]. The frozen duck feet were defrosted at 4-5 °C overnight in a refrigerator. After which, the were cut into smaller sizes and washed under tap water to get rid of blood and fat that were visible. The duck feet were soaked in 4.0% acetic acid at a ratio of 1:3 in a beaker for 1 hours. They were then neutralized to a pH of 5.5 by washing under tap water and subjected to hot extraction in a beaker containing distilled water (1:2 w/v) at 55°C for 12 hours in a waterbath (Memmert, WNB 22 Waterbath, Germany). After the extraction process, the layer of fat on the surface of the solution was disposed of, sieved and filtered with Whatman No. 4 filter. It was then transferred into thin layers on plastic trays and (Memmert, UN55 Universal Oven, Germany) dried at 50 °C for 12 hours until moisture content reached 80%. Lastly, the gelatin sheets were ground (Panasonic, MX-801S, Japan) into gelatin powder and stored at ambient temperature in a sealed container.

C. Colour Determination

Colour determination was carried out with Konica Minolta, Chroma Meter CR-400, Japan. This measured b*(+b*, yellowness/ -b*, blueness), a*(+a*, redness/ -a*, greenness) and L*(lightness) [17]. Chroma meter was calibrated before the measurements were taken and whiteness was determined by calculation [18]. The obtained gelatin powder was dissolved in distilled water at 60°C and left at temperature of 10°C for 16-18 hours for gel maturation process to produce gelatin gels.

D. Melting Temperature

Gelatin at 6.67% w/v concentration was used for the measurement of melting point. Melting temperature determination was done according to [19]. Twenty (20) mg gelatin gels was kept in 40 µL aluminum hermetically sealed pan and melted at a rate of 5–70 °C for 2°C/min.

E. Amino Acid Composition

Amino acid profiling was done using a slightly modified procedure of Nik Muhammed et al. [20]. The gelatin samples were digested at 110°C for 24 hours using 5 mL of 6N HCl in a sealed glass tube. Following this, 0.4 mL of alpha amino butyric acid (50 µmol /mL) was added to serve as internal standard. After that, distilled water (100 mL) was added to the aliquot and filtered using filter paper and syringe filter. Derivatizing reagent, borate buffer and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate were used to derived the samples. It was then separated with high performance liquid gas chromatography using eluent A (AccQ Taq concentrate, waters brand) and eluent B (Acetonitrile 60%, Sigma). The system consistent of: Waters 7171 auto-sampler, Waters binary 1525 HPLC pump and Waters satin box, Waters 2475 multi-fluorescence detector (excitation at 250 nm and emission at 395 nm), and Waters AccQ.Tag column (3.9 mm X 150 mm). The eluent flow was at a rate of 1mL/min. Peaks from the chromatography were combined, identified and measured using Breeze software version 3.20 and compared with known standard (Amino acid standard H; Pierce, Rockford, IL, USA)

F. Hydroxyproline Content

This was done according to AOAC [21]. 100µL sample was prepared in a test tube and 0.75 mL of 3.5M sulphuric acid was added. The test tube was placed in 105°C drying oven (Memmert, UN55 Universal Oven, Germany) for 16 hours. After 16 hours, water was added to the hot hydrolysate before being kept in 500 mL volumetric flask. was transferred to 500 mL volumetric flask. The sample was diluted to 12.5 mL with water. The final dilution (2.0 mL) was transferred into a test tube and distilled water (2.0 mL) was added. Oxidant solution (1 mL) was added to the test tubes, shook and allowed to stand for 20 minutes. After which, colour reagent (1.0 mL) was added, thoroughly mixed, capped and covered with aluminum foil. It was immediately placed in a waterbath (Memmert, WNB 22 Waterbath, Germany) and incubated at 60°C for 15 min. It was then placed on ice for 5 min to stop the reaction. The absorbance solution was measured using a visible spectrophotometer (Thermo Fisher, Thermo Spectronic Genesys 20, United States) at 558 nm. Standard solution was made from stock solution of 6 µg/ml to 0.6, 1.2, 1.8 and 2.4 µg hydroxyproline/ml. Hydroxyproline content was calculated according to the formula below:

Hydroxyproline content, g/100g = (hydroxyproline filtrate x 2.5)/(weight of test portion x volume of filtrate for dilution).

G. Data Analysis

Each sample was run in triplicate. All data collected were analysed by using SPSS (Statistical Package for Social Science) software version 25.0 (SPSS Inc., Chicago, II, U.S.A). Comparison of means among sample were calculated using Duncan's multiple range tests at a significant level of $p < 0.05$.

III. Result and Discussion

A. Colour Determination

Table 1 shows that the L* value (lightness) of KCDG and MDFG were significantly different from CBG and PDFG. L* values of the gelatin samples were 20.57, 17.85, 26.15 and

21.41 for KCDFG, MDFG, CBG and PDFG, respectively. Commercial Bovine Gelatin shows a higher L* value than Duck Feet Gelatin. PDFG had the lightest colour among all the duck feet gelatins, followed by KCDFG and MDFG.

TABLE I
L*, *A, AND B* OF THREE DIFFERENT BREED OF DUCK FEET GELATIN (DFG) AND COMMERCIAL BOVINE GELATIN (CBG)

Sample	L*	a*	b*	Whiteness
CBG	26.15 ± 2.55^{b}	-0.90 ± 0.05^{b}	5.02 ± 0.08^{a}	25.97 ± 2.54^{b}
KCDFG	20.57 ± 3.08^{a}	-0.05 ± 0.06^{d}	9.47 ± 0.60^{c}	19.99 ± 3.00^{a}
MDFG	17.85 ± 0.60^{a}	-1.49 ± 0.03^{a}	8.57 ± 0.03^{c}	17.38 ± 0.60^{a}
PDFG	21.41 ± 0.09^{b}	-0.05 ± 0.15^{c}	6.17 ± 0.36^{b}	20.82 ± 0.07^{a}

Means with the same superscript letters within the same columns are not significantly different at $p > 0.05$ and vice versa. KCDFG, Khaki Campbell duck feet gelatin; MDFG, Muscovy duck feet gelatin; PDFG, Pekin duck feet gelatin; CBG, commercial bovine gelatin

For a* (redness) values, there were significant differences ($p < 0.05$) among the samples and the values were -0.90 (CBG), -0.05 (KCDFG), -1.49 (MDFG) and -0.05 (PDFG). KCDFG and PDFG had a higher intensity of red colour compared to MDFG and CBG. Meanwhile, the b* values showed that KCDFG (9.47) was significantly higher ($p > 0.05$) than MDFG (8.57), PDFG (6.17) and CBG (5.02). Commercial bovine gelatin had a whiteness value that was significantly different ($p < 0.05$) from all the duck feet gelatins. The whiteness values for the gelatin samples were 25.97, 19.99, 17.38 and 20.82 for CBG, KCDFG, MDFG and PDFG, respectively. Whiteness of CBG was significantly higher ($p > 0.05$) than the other three duck feet gelatins.

Kuan et al. [16] found that, the L*, a* and b* values of duck feet gelatin extracted with 4% acetic acid were 17.44, -0.99 and -1.20, respectively. Broiler skin gelatin that had undergone an alkaline treatment process using 0.15% sodium hydroxide had L* values, a* values and b* values of 27.62, -2.98, and 1.33, respectively [22]. Besides that, quail bone gelatin extracted using 0.1M citric acid had L* values, a* values and b* values of 33.34, -2.19, 4.55, respectively [23].

Commercial gelatin usually has color range from yellow to dark amber [24]. DFG that was extracted in this study showed colour qualities that were similar to commercial gelatin. Gelatin samples have different colour values which is affected by the raw material used during extraction and type of acid used [25, 26]. This can be affected by mucosubstance, inorganic contaminants and proteinaceous substances introduced or removed during the extraction of gelatin [27]. Also, colour is a very important parameter of gelatin that affects its acceptability and the functional property [28]. Kuan et al. [16] also indicated that, generally, for physical appearance of gelatin, colour is one of the important qualities looked for as well as its none functional properties in food products

B. Melting Temperature

Figure 1 shows the melting temperature of gelatin samples. There were significant differences ($p > 0.05$) among the melting temperatures for the CBG, KCDFG, MDFG and PDFG at with the value 36.77°C, 61.91°C, 48.62°C and 44.81°C accordingly.

Figure 1 shows the melting temperature of the gelatin samples. There were significant differences in melting temperature among the duck feet gelatins. KCDFG (61.91°C), was significantly higher ($p > 0.05$) than CBG (36.77°C), MDFG (48.62°C) and PDFG (44.81°C). Thus KCDFG had the highest melting temperature of 61.91°C, and PDFG had the lowest melting temperature of 44.81°C. Commercial bovine gelatin had lower melting temperature than all the duck feet gelatins. Furthermore, gelatin with a higher critical concentration and a lower melting point will have a lower imino acid content. Gelatin with a lower melting point and higher critical concentration had a lower imino acid content compared to gelatin with high imino acid content [28, 29]. Nevertheless, the imino acid content of commercial bovine gelatin is higher than duck feet gelatin. The imino acid content of CBG was 25.65%, followed by PDFG (18.63%), KCDFG (16.76%) and MDFG (16.23%).

Gelatin melting temperature is the temperature where the gelatin gel becomes soft. It is one of the significant physical attributes that can be used to measure the gelatin quality [23, 30]. Bichukale et al. [31] reported that chicken skin gelatin that has undergone alkaline extraction using 0.15% sodium hydroxide at 55°C had a melting point of 32.60°C, and the melting point of poultry bone that was extracted with 4% hydrochloric acid at 55°C had a melting point of 29.87°C. In addition, Samsudin et al. [23] showed that the melting point for quail bone gelatin extracted with o.1 M citric acid at 75°C was 34.50°C. Duck feet gelatin had a higher melting point compared to the all reported gelatins from poultry.

Pranoto et al. [32] reported that, gelatin extracted from five-lined threadfin bream (*Nemipterus tambuloides*) skin using 0.05M acetic acid at 80°C had a melting point of 31.67°C. Besides that, melting point of other fishes skin such as Alaska pollock (*Theragra chalcogramma*) [33], Silver carp (*Hypophthalmichthys molitrix*) [34], Rainbow trout (*Oncorhynchus mykiss*) [35] and Yellowfin tuna (*Thunnus albacares*) [36] were 21.2°C, 29°C, 23°C and 24.3°C, respectively [24]. Duck feet gelatin had a higher melting point compared to fish skin gelatin. Mammalian collagen has a higher melting point and gel strength than marine collagen due to a higher amount of proline and hydroxyproline (imino acids) [37, 38].

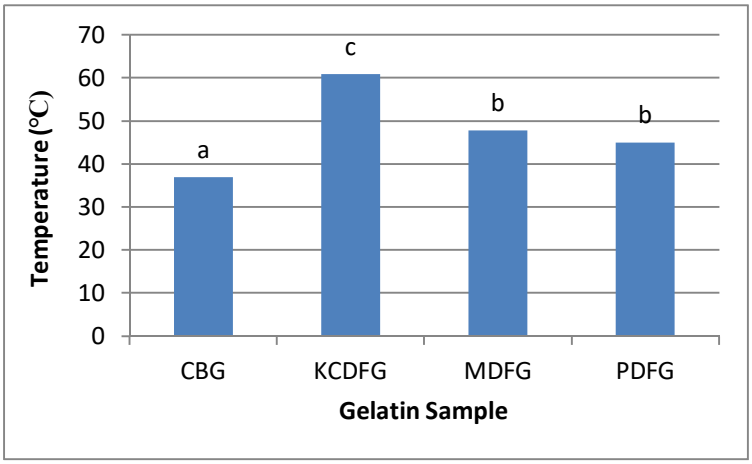

Fig. 1 Melting temperature of three different breed of duck feet gelatin (DFG) and commercial bovine gelatin (CBG)

C. Amino Acid Composition

Table 2 shows the amino acid content of CBG, KCDFG, MDFG and PDFG. The results showed that, the main amino acids present are glycine (21.73%, 13.62%, 13.46% and 15.33%), proline (13.08%, 8.71%, 8.39% and 9.34%), hydroxyproline (12.57%, 8.05%, 7.84% and 9.39%) and alanine (8.79%, 5.77%, 6.03%, 6.65%) for CBG, KCDFG, MDFG and PDFG, respectively. Glycine is one of the major amino acids in gelatin [39, 16].

TABLE II
AMINO ACID CONTENT OF THREE DIFFERENT BREED OF DUCK FEET GELATIN (DFG) AND COMMERCIAL BOVINE GELATIN (CBG)

Amino acids (%)	CBG	KCDFG	MDFG	PDFG
Alanine	8.79	5.77	6.03	6.65
Arginine	6.96	4.88	5.06	5.56
Aspartic acid	5.50	4.39	3.96	4.34
Glutamic acid	9.94	7.64	6.92	7.54
Glycine	21.73	13.62	13.46	15.33
Histidine	0.67	0.74	0.63	0.80
Hydroxyproline	12.57	8.05	7.84	9.39
Isoleucine	1.46	1.61	1.26	1.23
Leucine	2.90	3.16	2.55	2.57
Lysine	3.61	3.12	2.99	2.81
Methionine	1.40	1.24	1.02	1.10
Phenylalanine	1.84	1.38	1.63	1.70
Proline	13.08	8.71	8.39	9.24
Serine	3.07	2.16	1.90	2.21
Threonine	1.91	1.76	1.51	1.70
Tyrosine	0.21	0.54	0.50	0.43
Valine	2.50	2.17	1.79	1.82
Imino Acid	25.65	16.76	16.23	18.63

Meanwhile, for proline content, PDFG had the highest percentage of amino acid compared to the other two DFG, but lower than CBG. The proline content of the gelatin in this study were 13.08%, 9.24%, 8.71% and 8.39% for CBG, PDFG, KCDG and MDFG, respectively. A previous study by Kuan et al. [16] reported that the proline content of PDFG extracted by using 4.0% v/v acetic acid was higher (11.60%) compared to this study (9.24%). Another study by Nik Muhammad et al. [20] also found a higher proline content (12.23 %) of duck feet gelatin extracted with 0.1M acetic acid. Commercial bovine gelatin and duck feet gelatin hydrolysate from three different breeds of duck also contain a higher content of arginine and glutamic acid. This result agrees with the finding from Abedinia et al. [40], where Pekin duck feet gelatin extracted by using acid, alkali and enzyme had a higher composition of arginine (56.73%, 59.64% and 63.22%) and glutamic acid (55.37%, 59.49%, 60.77% and 54.14%), respectively.

On the other hand, the lowest composition of amino acid contents were tyrosine, histidine, methionine and isoleucine in all gelatin samples studied with no trace of cysteine. The presence of cysteine in the gelatin sample is caused by contamination during the gelatin extraction process by non-collagenous proteins [20]. Tyrosine and histidine contents are very low in both CBG and DFG. Kuan et al. [16] stated that cysteine and tryptophan are not usually found in gelatin samples, thus in agreement with this current study. The gelatin sample in this study can be categorized as type I collagen due to the absence of cysteine and tryptophan [22]. All the duck feet gelatins from three different breeds had undergone a clean extraction process and were identified as type I collagen.

Proline and hydroxyproline amino acids, also known as imino acid, are the main component for the construction of stable triple helix structure. Triple helix structure is significant in forming hydrogen bonds between hydroxyl groups of hydroxyproline in gelatin and free water molecules [26, 41]. Half of the α-chain collagen is made of tripeptides that have the general formula of glycine –XY, where X is usually proline, and Y is commonly hydroxyproline. The stabilization of the triple-stranded collagen helix is mainly affected by hydroxyproline due to its hydrogen bonding ability with the hydroxyl group [41]. Gelatin extracted from difference sources have the entire 20 amino acids, yet distinguished amino acid composition [16]. Amino acid composition and the molecular weight distribution have a vast impact on the gelatin functional properties [42].

Nik Muhammad et al. [20] reported that the amino acid composition of duck feet gelatin extracted by using 0.1M acetic acid had a high percentage of glycine, proline and hydroxyproline values which were 29.04%, 12.23% and 10.31%, respectively. Meanwhile, the amino acid composition of chicken feet extracted with 1.5% acetic acid had 31.51% glycine, 17.60% proline and 9.24% hydroxyproline [26]. Broiler skin that had undergone an alkaline extraction process using 0.15% sodium hydroxide also contains amino acid with a dominant composition of glycine (20.26%), proline (15.12%) and hydroxyproline (11.36%) [22]. The amino acid composition of PDFG in this study was 15.33% glycine, 9.34% proline and 9.39% hydroxyproline. The finding is comparable to other gelatin study.

D. Hydroxyproline Content

Table 3 shows the hydroxyproline content (g/100g) of CBG, KCDFG, MDFG and PDFG. The hydroxyproline content were 12.04, 8.00, 7.63 and 9.15 g/100g for CBG, KCDFG, MDFG and PDFG, respectively. There were significant differences ($p < 0.05$) between duck feet gelatin hydrolysate and commercial bovine gelatin hydrolysate.

TABLE III
HYDROXYPROLINE CONTENT OF THREE DIFFERENT BREED OF DUCK FEET GELATIN (DFG) AND COMMERCIAL BOVINE GELATIN (CBG)

Sample	CBG	KCDFG	MDFG	PDFG
Hydroxyproline Content (g/100g)	12.04 ± 0.09^{c}	8.00 ± 0.07^{b}	7.63 ± 0.13^{a}	9.15 ± 0.04^{d}

Means with the same superscript letters within the same columns are not significantly different at $p > 0.05$ and vice versa. KCDFG, Khaki Campbell duck feet gelatin; MDFG, Muscovy duck feet gelatin; PDFG, Pekin duck feet gelatin; CBG, commercial bovine gelatin.

Hydroxyproline content range from 8.62g/100g to 9.10g/100g for duck feet gelatin, which is lower than commercial bovine gelatin (11.04 g/100g). Nevertheless, the value is higher than the hydroxyproline content of chicken feet gelatin extracted using 4.5% acetic acid (6.36g/100g) reported by Chakka et al. [22]. Lee et al. [43] proposed that the main amino acid component of gelatin from duck skin that had undergone acid and alkali pretreatment were hydroxyproline and glycine [40]. The only protein that is made up of considerable amounts of hydroxyproline is gelatin [16]. Hydroxyproline value positively influences bloom strength of gelatins. A greater amount of hydroxyproline content will result in an excellent bloom strength [26]. PDFG (139.87 bloom) had a higher bloom strength than KCDFG (63.78 bloom), but lower than CBG (150.71 bloom).

IV. CONCLUSION

Pekin duck feet gelatin had the lightest colour among all the duck feet gelatins, followed by Khaki Campbell and Muscovy duck feet gelatin. The major composition of amino acid in duck feet gelatin are glycine, proline and hydroxyproline which is comparable to commercial bovine gelatin. The hydroxyproline composition for the duck feet gelatin is comparable to both amino acid profiling and hydroxyproline content. This study showed that the physiocochemical properties of duck feet gelatin had similar properties as the commercial bovine gelatin. Duck feet gelatin can be a potential raw material and as an alternative for commercial gelatin that can be used in food industry.

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