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ORIGINAL ARTICLE

Extraction of Gelatin from Different Parts of *Gallus Gallus Domesticus*

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ABSTRACT – Gelatin is a mixture of protein obtained from animal parts (skin, bones, tendons, ligaments, and cartilages) by hydrolysis. Gelatin market demand is high especially in pharmaceutical, food, photographic, and cosmetics industries based on its gelling, foaming, and emulsifying properties. This preliminary study was focus on chicken head and feet gelatin extraction and their characterization in terms of percentage yield and physical properties (pH, color, melting point, and texture). Two different treatments (acid and alkali) were used. The percentage yield of chicken head (CHGac) and feet (CFGac) gelatins by using acid treatment were 32.10% and 33.65% while the chicken head (CHGal) and feet (CFGal) from alkali treatment were 20.06% and 22.18% respectively. All the gelatins indicated the same pH pattern range from 4.3 to 6.4. The melting point for gelatins from each treatment was a range from 30.4°C to 35.9°C. The texture analysis is specifically into the gel strength of gelatin produce which ranges from 230 g to 356 g. All the gelatins also showed the same pattern of dl(lighter/darker), da(redder/greener), and db(yellower/bluer) which are positive value mean that the gelatins have lighter, redder and yellower color respectively. In conclusion, chicken head and feet can be an alternative that could replace the usage of porcine, bovine, and other mammals as a source of gelatin.

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INTRODUCTION

The poultry industry in Malaysia is one of the highest demand food sectors with an estimated consumption in 2020 is up to 49.3 kg of poultry meat per capita. As an essential protein source in Malaysia with an average annual production (2013-2019) of 1638 million tons of chicken meat, it is denoted that, 0.82% of enhancement in production of broilers in 2019 [1]. The rapid development of this industry has led to an abundance of by-products produced during the slaughtering processes. Consequently, these by-products will result in environmental problems such as soil pollution, air pollution, pathogens, heavy metals, and water quality problems [2] [3]. However, if these abundant by-products are fully utilized with an innovative idea with the aid of technology, it will certainly contribute to economic growth and give the value-added to the entire poultry industry chain without affecting the environment. By implementing the Sustainable Development Goals (SDGs), the socioeconomic and sustainability environment could be achieved by utilizing the by-products from the poultry industry (head and feet) to generate value-added products applicable to pharmaceutical, cosmetics, food, and tissue engineering industries [4] [5]. The chicken head that is commonly used in animal feed is rich in collagen mainly in a comb, wattle, skin, bones, and cartilages able to produce high bloom strength of Type-A gelatin that higher than bovine gelatin [6]. The same goes for chicken feet, this waste product received special attention due to nutritional content that gives benefits to human health and value-added to food, cosmetics, and pharmaceutical industries because of its capability to form a gel, bind, and act as an emulsifier. Both types of gelatin (A and B) have different physicochemical properties that lead to different applications [7]. Since the chicken head is a homogenous tissue combination of skin and bone, therefore, the acid and alkali pre-treatment method is applied and basic physical characteristics (pH, melting point, color, viscosity, bloom strength) of different parts of Gallus gallus domesticus (head and feet) were compared with the commercial bovine gelatin (CBG).

MATERIALS AND METHODS

***Gallus gallus domesticus* preparation**. Fresh chicken head (CH) and feet (CF) were obtained from Yasin & Al-Haj Enterprise at Beserah Kuantan, Malaysia. Once received, all parts were cleaned and weighed for 5 kg each using Shimadzu digital balance and recorded. Then, the cleaned samples were ground using a Philips food processor (HR77629). The ground samples were vacuum packed and stored in a freezer at -20°C before being analyzed.

Defatted process. The defatting process of chicken head (CH) and feet (CF) were conducted using a cold extraction technique [8]. In this process, ethanol (C2H5OH) and petroleum ether (C6H14) were mixed with a ratio of 1:1. Then the ground chicken head and feet were homogenized for 6 h with the 1:1 ethanol-pet ether according to 1:6 (w/v) ratios. The

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homogenized solution was collected using Edwards vacuum filter, UK, and transferred into the desiccator till the filtrate dry.

Gelatin pre-treatment and extraction

Acid-soluble extraction. The defatted chicken feet (CF) and head (CH) were soaked, shaken, stirred, and stored at room temperature in 5% (v/v) lactic acid (C3H6O3) solution for three days where this pre-treatment method was adapted and modified from [9]. The chicken feet and head were properly and completely immersed in the lactic acid (C3H6O3) solution to remove the non-collagenous material. After three days, the lactic acid (C3H6O3) solution was removed and drained from the chicken feet and head followed by a neutralization process by adding 0.2M (1:4 w/v) sodium hydroxide (NaOH) until a pH of 7 was reached. The mixture of the samples was centrifuged for 60 min before undergoing hot water extraction at 85 °C for 1 h before filtered by using vacuum filtration. The extracted gelatin was freeze-dried by laboratory freeze dryer Crist, the UK then kept for analysis.

Alkali-soluble extraction. 100 ml of distilled water was added into defatted chicken feet (CF) and head (CH) and centrifuged for 20 min. Then, 0.15% of sodium hydroxide (NaOH) was added into a defatted solution by centrifuging for 40 min and repeated for 3 cycles. 0.7% citric acid was then added to the supernatant and continues for centrifugation for 10 min. This method was carried out for 3 cycles by referring to [10] with several modifications. The supernatant was added with 100ml of distilled and continue to centrifuge for 10 min and let it cool for 15 min. After cooling, 4 g of activated carbon was mixed and filtered after 20 min using vacuum filtration. The filtered supernatants were undergoing hot water extraction at 85°C for 1 h and the concentrated chicken head gelatin (CHG) and chicken feet gelatin (CFG) solutions were dried in a laboratory freeze dryer Crist, the UK.

Figure 1 simplifies the treatment process using different methods and parts of Gallus gallus domesticus.

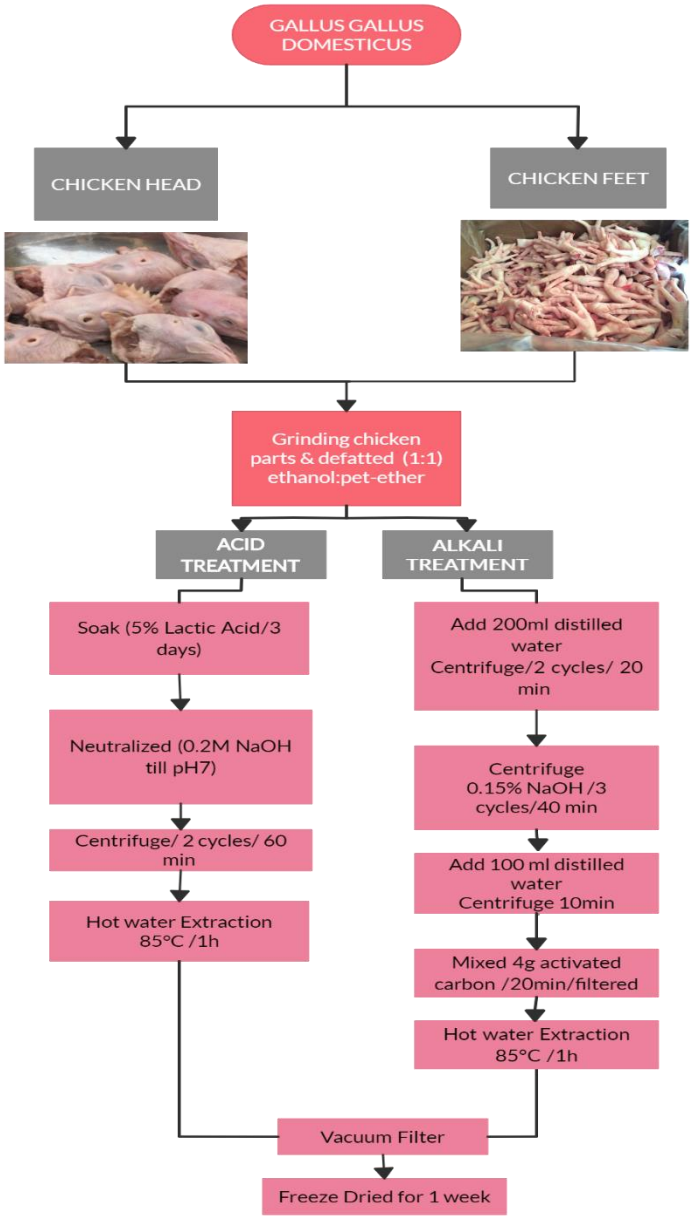

Figure 1 Treatment process using different methods and parts of *Gallus gallus domesticus*

Physical Analysis

Percentage yield determination. The percentage yield of gelatin was conducted by referring to [11]. The extraction yields of the gelatin were calculated based on the dry basis equation.

$$\text{Yield (\%)} = \frac{\text{Dry weight of gelatine (g)}}{\text{Dry weight of chicken parts (g)}} \times 100\% \quad (1)$$

Gel strength measurement. Gel strength analysis was conducted by referring to [11]. The bloom strength was measured by using Brookfield CT3 Analyzer, the USA with a 12.7 mm cylindrical plane surface and a sharp edge probe (AAC standard for bloom test). The speed was set up at 1 mm/s with a 5 mm targeted distance.

Color measurement. The CHG and CFG were measured using a calibrated Hunter Lab Color Quest XE spectrophotometer. The symbols L*, a* and b* indicated dl(lighter/darker), da(redder/greener), and db(yellower/bluer) of samples, respectively.

pH measurement. The pH value for CHG and CFG solution was measured with Milwaukee MW102 PRO, the USA based on [11]. 7.14% (w/v) of the gelatin solution was placed in a water bath for 15 min at 65°C. Two-point calibration was performed in buffer solutions at pH 4 and pH7.

Melting point measurement. The glass capillary tube was prepared for melting point gelatins determination. The capillary tube was inserted into the melting point apparatus. The temperature was set at 25ºC and the heating rate was programmed where the temperature will increase 1ºC for every 30 seconds. The melting point was observed through a viewfinder and recorded once the CHG and CFG powder melted.

Viscosity measurement. 10% (w/v) of the gelatin solution was heated at 60°C before measuring the viscosity for CHG and CFG solution with Brookfield Laboratory Viscometer base on [12] by using No. 1 spindle and rotated at 60 rpm.

Statistical analysis. SPSS Statistics Version 17.0 was used [11] to determine the significant difference with mean values of $p < 0.05$ for different parts and methods of gelatin.

RESULTS AND DISCUSSION

Chicken Head Gelatin (CHG) and Chicken Feet Gelatin (CFG) percentage yield. Gelatin percentage yield from both methods: Chicken Head Gelatin (CHG) and Chicken Feet Gelatin (CFG) represented by a mass of dry weight gelatin per total mass of the chicken part. From Table 1, the percentage yield of CHG and CFG with acid-soluble extraction was slightly higher compared to the alkali-soluble extraction method. The highest percentage yield was obtained from CFGac acid-soluble extraction (33.65%) may due to longer pre-treatment time compare to alkali-soluble extraction. The extraction yield not only depended on the types of chemicals used but they are also depended on types of raw materials, animal age, collagen content, chemical concentration, time of treatments and extraction, extraction temperature, and method of extraction [13] and [14]. The extracted gelatin for CHG and CFG in both extraction methods were in the range of 20.06% to 33.65% based on the dry weight.

Table 1 Characteristics of different methods and parts of Gallus gallus domesticus extracted gelatins

Characteristics	Acid-soluble extraction		Alkali-soluble extraction		Commercial Bovine Gelatin (CBG)
	CHG^{ac}	CFG^{ac}	CHG^{al}	CFG^{al}	
Percentage yield (%)	32.10±0.45 ^{b}	33.65±1.30 ^{b}	20.06±0.36 ^{a}	22.18±1.08 ^{a}	-
Bloom Strength (g)	320.00±0.10^{b}	356.00±1.00^{b}	230.12±0.30^{a}	268.00±1.10^{a}	152±0.60^{c}
Viscosity (cP)	4.49±0.15 ^{a}	4.38±0.67 ^{a}	3.52±0.22 ^{b}	3.35±0.13 ^{b}	4.10±0.18 ^{c}
Melting Point (ºC)	32.3±0.70^{a}	35.9±1.10^{a}	30.4±0.05^{b}	31.6±1.08^{a}	34.5±0.20^{a}
pH	4.3±0.10^{a}	4.8±0.04^{a}	6.2±0.06^{b}	6.4±1.00^{b}	5.3±1.20^{c}
Colour					
L*	62.12±0.03^{a}	53.22±0.45^{b}	55.11±0.12^{b}	59.29±0.19^{b}	52.29±0.13^{b}
a*	1.73±0.22^{a}	0.17±0.20^{b}	2.01±0.80^{c}	2.74±0.10^{c}	1.27±0.62^{a}
b*	15.35±1.90^{a}	17.93±0.08^{b}	16.90±0.56^{c}	17.13±0.65^{b}	16.11±0.42^{c}

CHG^{ac}: Chicken Head Gelatin with acid-soluble extraction method, CFG^{ac}: Chicken Feet Gelatin with acid-soluble extraction method, CHG^{al}: Chicken Head Gelatin with alkali-soluble extraction method, CFG^{al}: Chicken Feet Gelatin with alkali-soluble extraction method. This table represents mean ± standard deviation values. $^{a-c}$ in the same row designate significant difference ($P > 0.05$)

From analytical data, it shows that the acid and alkali pre-treatment with different chicken parts gave significantly different ($p>0.05$) on the percentage yield of Gallus gallus domesticus. The previous study from [15],[16], and [17] were using acid and alkali pre-treatment extraction process shows the percentage yield for chicken feet gelatins were in the range of 10.29% to 30.04% while for chicken head gelatins by [18] and [19] were 16% and 31.21% which were slightly

lower than this finding. From the result in table 1, it can be observed that the defatted and improvement of pre-treatment extraction techniques for CHG and CFG aids in the percentage yields for both methods, and the alkali-soluble extraction method can be improvised in terms of the pre-treatment cycle to complete the gelatin hydrolysis thus gain the higher percentage yield of gelatin.

Gel strength. Gel strength that is measured with the Bloom test ranges from high (300g) to low (50g) represent the quality of gel [20]. Different product applications will require certain Bloom of gelatin. In this study, the commercial bovine gelatin (CBG) had the lowest gel strength (152g) compare to CHGac (320g), CFGac(356g), CHGal(230g), CFGal(268g). The gel strengths in CHGac, CFGac were significantly different ($p < 0.05$) compare to CHGal, CFGal. Lower gel strength in CHGal, CFGal might due to shorter extraction time which resulted in incomplete hydrolysis of gelatin. This partial hydrolysis results in weakened peptide bone and fewer the α-chains consequently, unable to form a stronger molecular bond. Vice versa, high gel strength in CHGac, CFGac were comparable to chicken skin gelatin reported by [21] and higher than gel strength in porcine [22]. A previous study by [19] shows, lower Bloom of chicken head gelatins from 200.4 g to 247.9 g were obtained and the gel strength from aforementioned [14], [16], and [21] work were comparable to CHGal, CFGal but lower than CHGac, CFGac. Overall, the CBG and all the extracted gelatins were applied to food industries as the gel strengths were in the range of medium to high Bloom.

Colour. Table 1 indicated the color of CHG and CFG under different pretreatment methods which represented as L*(lighter/darker), a*(redder/greener), and b*(yellower/bluer). The color attributes for CHG and CFG have significant differences ($p< 0.05$) when subjected to different extraction methods. The raw material and treatment method resulted in color difference and consumer acceptance [23]. The values of L* and b* of CFGal and CBG were lower compare with CFGac, CHGac, and CHGal. The lower L* values were probably due to the longer pre-treatment time as mention by [24]. According to [25], a longer pre-treatment time will result in non-enzymatic browning leading to a decrease of L* values. For a* value, only CFGac was greener than redder and there was a significant difference in b* value for the CHGac towards other extracted gelatin where all of them were more to yellower in color. In brief, the CFGac, CHGac, and CHGal were lighter in color (higher L* value) was preferable than the CFGac alkali-soluble treatment as supported by [26].

***p*H.** The types of chemicals and concentrations being subjected to the raw materials will influence the gelatin pH [27]. The range value for pH 3.8 to 5.0 was for type-A gelatins that undergo acid pre-treatment while for type-B gelatin that is subjected to alkali pre-treatment, the range value was pH 4.7–7.5 [28]. From table 1, the pH of CHGac and CFGac that undergo acid-soluble treatment were 4.3 and 4.8 respectively, while pHs for alkali-soluble treatment (CHGal and CFGal) were 6.2 and 6.4 respectively. The pH values for CHGal and CFGal were close to neutral due to the alkali pre-treatment in the extraction process and the addition of distilled water in each cycle with subjected to carbon filtration before undergoing hot water extraction. The difference in pH between CBG and CHGac, CFGac, CHGal, CFGal was found to be not significant ($P<0.05$). From the result, it can be concluded that CHGac and CFGac were type-A gelatin and CHGal and CFGal were type-B gelatin with high Bloom strength. Concisely, the pH range influence by the concentration of the chemical used before pre-treatment and extraction. The pre-treatment step is one of the major factors that influence the pH gelatins.

Melting Point. The melting temperatures for CHGal and CFGal (30.4°C and 31.6°C) were lower than CHGac and CFGac (32.3°C and 35.9°C). However, no significant differences ($p < 0.05$) were found between the melting point of CBG and CHGac, CFGac, CHGal, CFGal. The melting point is measured once the gel started to melt which results in decreasing in carbon tetrachloride [29] and this process is connected with Bloom strength and viscosity of gelatin [29]. [29] and [30] mentioned that the melting point of gelatin is influenced by proline and hydroxyproline content. These amino acids assist the collagen stability where hydroxyproline and proline content is proportional to the melting temperature. Therefore, further research can be conducted to determine the hydroxyproline content in CHGac, CFGac, CHGal, CFGal.

Viscosity. Viscosity is conducted to determine the gelatin quality [31]. From the results, the viscosities of CHGac and CFGac extracted from acid pre-treatment were 4.49 and 4.38 cP, whereas the CHGal and CFGal that extracted from alkali pre-treatment were 3.52 and 3.35cP. Generally, pH, the molecular weight of protein constituents with their distribution will result in differences in gelatins viscosities [32][33]. However, no significant differences ($p < 0.05$) were found between CBG and CHGac, CFGac, CHGal, CFGal indicated that these gelatins were applicable for commercialization even though most commercial gelatins viscosities could attain higher than 4.0 cP and may up to 13.0 cP [34]. Due to the high temperature (85°C) during gelatin extraction, the energy was absorbed by water molecules which result in a declination of CHGac, CFGac, CHGal, and CFGal viscosities. Based on [35], temperature and time extraction were inversely proportional to viscosity. Therefore, the higher and longer the extraction temperature and time of gelatin, the lower value of viscosity obtained which results in low quality of gelatin produced.

CONCLUSION

From this preliminary research, basic physical characteristics (pH, melting point, color, viscosity, bloom strength) of different parts of Gallus gallus domesticus CHGac, CFGac, CHGal, and CFGal were determined and effectively extracted. Different pre-treatment methods define the types of extracted gelatin as CHGac and CFGac indicated Type-A gelatin otherwise CHGal and CFGal showed Type-B gelatin which was comparable to the commercial bovine gelatin (pH, color, melting point, and viscosity) and higher in gel strength (230-320) g. In brief, further physicochemical analysis was highly

recommended to CHGac, CFGac, CHGal, and CFGal as they have a huge potential to replace porcine, bovine, and other mammals' gelatin in various industrial applications.

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