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Preliminary Study on Physicochemical Properties of Duck Feet Collagen

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Abstract: Duck feet collagen was extracted using 5% lactic acid and examined for their physicochemical properties (chemical composition and amino acid, yield, pH and swelling percentage, color and Fourier Transform Infrared (FTIR) spectroscopy). Chemical composition of duck feet collagen such as moisture, protein, fat and ash content was 5.85, 29.11, 35.43 and 28.60%, respectively. 17 amino acids were detected in duck feet collagen and included 20.46% glysine, 7.73% hydroxyproline and 10.24% proline. The yield of collagen obtained from this treatment was 28.37%. The collagen extracted was light in color with a pH 2.67 (soaking period) and the swelling percentage was 240.50%. Duck feet collagen (DC) possessed similar bands (Amide A, Amide I, Amide II and Amide III) with commercial Fish Collagen (FC) and commercial Cow Collagen (CC) for the FTIR.

Key words: Collagen, duck feet, physicochemical properties, amino acid composition

INTRODUCTION

Collagen constitutes about 25-30% of total bodily protein complement. It is the main and most abundant mammals' protein forming the major protein constituents of skin, tendon, cartilage, bone and connective tissue. Collagen has very broad application in various branch of industry such as materials in foods, cosmetic and pharmaceutical industry (Rodziewicz *et al*., 2008). Collagen can also be used for capsulation and edible film formation in biomaterial-based packaging (Benjakul *et al*., 2005). The properties of collagen normally help some food products to have higher quality when collagen is added to such food products (Sadowska *et al*., 2003).

Mainly collagen sources are from mammal, fish and poultry. Pachence and Li (1992, 1993) stated that collagen in poultry feet are abundant and can be extracted to produce collagen film or collagen powder. Duck is one of the poultry type which is widely available in Malaysia. Although the total production of ducks is still less than that of chickens, duck production increased more rapidly (~400%) than chicken production (~250%) between 1996 to 2006 (DVS, MOA, 2008). The total production of ducks in Malaysia was 2,341,762 birds in 1996 and it increased to 9,361,108 birds in 2006. Malaysia is the third main producing country of duck meat in the world after China and France (Anonymous, 2011). The huge duck production in Malaysia means that a great deal of by-products (feets) is also produced and provide readily available source of raw material to produce duck collagen.

Previous studies on bird feet by Liu *et al*. (2001) and Cheng *et al*. (2009) have reported the possibility of extracting collagen from broiler chicken feet and silky fowl feet by using a variety of acids. Liu *et al*. (2001) discussed four acid extractions of collagen from broiler chicken feet. Cheng *et al*. (2009) also suggested that, it is possible to obtain collagen containing melanin from silky fowl feet using a variety of acids. The availability of duck feet presents an opportunity to expand its use into many further processed meat products. The aim of this study was to determine the physicochemical properties of collagen extracted from duck feet.

MATERIALS AND METHODS

The frozen duck feet were purchased from local duck food industries which is located in Northern part of Malaysia and stored at -20°C for further analysis. Lactic acid and sodium hydroxide were used in the extraction of duck feet collagen. Commercial fish scale collagen and cow collagen were purchased from local suppliers. Collagen was extracted with an acid extraction method as described by Liu *et al*. (2001). Duck feets were thawed in a chiller at 6°C for 24 hours. They were then cut into smaller pieces after the claws were removed and ground twice using a 10 mm plate mechanical mincer (Model EVE/ALL-12, Rheninghaus, Torino, Italy). To isolate the collagen duck feet paste, the ground duck feet were mixed with 5% lactic acid solution by w/v (duck feet/solution = 1/8) and soaked for 24 hours at 4-7°C. At the end of soaking, the layer of fat on the surface of solution was disposed off. Treated duck feet suspended

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solutions were homogenized by a blender (Panasonic, MX-799) for 5 min and then filtered by a double gauze to discard bone residues. These suspended solutions were neutralized to pH 7 with 1.0N NaOH. The neutral solutions were centrifuged with a high speed centrifuge (Model Union 5KR, Korea) at 5000g for 15 min at 10°C. The supernatant was discarded and the precipitate was lyophilized by a freeze dryer (LD53, Kingston, New York) to obtain dry collagen.

Chemical composition and amino acid: The chemical contents (moisture, protein, fat and ash) and amino acid composition of duck feet were determined by standard procedures of the Association of Official Analytical Chemists (AOAC, 2000). Hydrolysis for amino acid analysis were done by using 6 N HCl (except for cystine, methionine and tryptophan), performic acid (cystine and methionine) and 4.3N LiOH (tryptophan). The collagen was analyzed in duplicate with three hydrolyses. The amino acid content was determine by HPLC using a amino acid analysis column C-18 AccQ.TabTM (Waters, Ireland).

Yield, pH and swelling percentage: The yield (%) of crude collagen was calculated by the following formula -[weight of frozen dry crude collagen/weight of ground duck feet] x 100 (Liu *et al*., 2001). pH of crude collagen samples were measured at the end of soaking time according to the procedure of Ockerman (1984) by using pH Meter (Mettler Toledo Delta 320). According to the method of Liu *et al.* (2001), duck ground feets were filtered using a stainless filter for 15mins to obtain the weight of super-solid from filtered product at the end of soaking time. Swelling percentage was calculated utilizing: [weight of the super solid from filtered product after soaking/weight of ground duck feet soaking] x 100%.

Colour determination: The colour of crude collagen from duck feet was determined using colorimeter Minolta model CM-3500d spectrophotometer (Minolta, Kyoto, Japan) with Spectramagic Version 2.11, 1998 (Minolta) software. The method of colour measurement used is Commission Internationale de l'Eclairage (CIE) L*, a*, b*colour space which indicate lightness, redness and yellowness of sample in this study.

Fourier Transform Infrared (FTIR) spectroscopy : FTIR was done according to the method of Woo *et al.* (2008). FTIR spectra were obtained from 1mg collagen in approximately 100 mg potassium bromide (KBr). All spectra were obtained from 4000 to 500 cm^{-1} at a data acquisition rate of 2 cm^{-1} by using a FT-IR spectrophotometer (Nicolet iS10, USA).

Statistical analysis: Data obtained from all the analyses were analyzed using the statistical one-way analysis of variance (ANOVA), followed by Duncan multiple range test of Statistical Package for Social Science version 16.0 (SPSS inc., Chicago, Illinois, U.S.A). Statistical significance was established at 0.05 levels.

RESULTS AND DISCUSSION

The chemical composition and amino acid contents: The chemical composition of duck feet collagen and it comparison with commercial fish and cow collagen is presented in Table 1. The results showed that, duck feet collagen contained a lower protein content (29.11 %) than commercial fish collagen (89.17%) and commercial cow collagen (91.70%). High content of fish collagen protein was also reported by Kittiphattanabawon *et al.* (2005) on bigeye snapper fish bone collagen (84.2%) and bigeye snapper fish skin collagen (94.0%). Fat content of duck feet collagen (35.84%) was much higher than commercial fish collagen (0.13%) and commercial cow collagen (0.07%). Kittiphattanabawon *et al.* (2005) also reported a lower fat content for bigeye snapper skin collagen (0.33%) and bone collagen (0.48%). The ash content of duck feet collagen (28.60%) was also much higher than commercial fish collagen (7.31%) and commercial cow collagen (0.35%). High level of fat content in duck feet collagen was due to the absence of defatting process in this experiment, while for ash content, it could be associated with the soaking time of duck feet in lactic acid during preparation.

The amino acid composition of duck feet collagen is shown in Table 2. Present study found 17 amino acids and histidine and cysteine were not detected. Liu *et al.* (2001) reported that no tryptophan and hydroxyproline were detected in chicken feet collagen. Glysine was the dominant amino acid found in duck feet collagen with 20.46%. However, it was lower compared to chicken feet collagen which contained 27.84 % (Liu *et al*., 2001). Hydroxyproline is an amino acid obtained from proline (Norziah *et al*., 2009). The amino acids (hydroxyproline and proline) of duck feet collagen was 17.97% and in agreement with amino acid content of interstitial collagen (approximately 17%) reported by Gomez-Guillen *et al.* (2002). Both amino acid and glycine play an important role in gel strength (Wangtueai and Noomhorm, 2009). Low amino acids content also indicates poor gelling power (Ward and Courts, 1977). Absence or low amounts of cysteine (~0.2%) and methionine (~1.24-1.33%) are usually found in type I collagen (Pati *et al*., 2010; Singh *et al*., 2011). Only collagen types III, IV and CP55 contained cysteine and methionine. Hence, duck feet collagen contains type I collagen as it had low cysteine and methionine content detected in only 1.77%.

Yield, pH and swelling percentage: The yield, pH and swelling percentage of duck feet collagen are shown in

Table 1: Chemical composition of duck feet collagen, commercial fish collagen and commercial cow collagen+.

	Moisture (%)	Protein (%)	Fat (%)	Total Ash (%)
Duck feet collagen	5.85±0.48	29.11±0.14	35.43±0.44	28.60±0.20
Commercial fish collagen	2.48±0.16	89.17±4.51	0.13±0.06	7.31±0.10
Commercial cow collagen	8.08±0.01	91.70±1.05	0.07±0.01	0.35±0.03

n = 3; +Values are Mean±Standard deviation

Table 2: Amino acids content of the collagen obtained from duck feet and chicken feet

		Collagen sample	
Amino acid (%)		Duck feet	+Chicken feet
Histidine	His	ND	0.99
Isoleusine	Ile	2.43	2.27
Leucine	Leu	4.69	4.35
Lysine	Lys	5.41	4.05
Methionine	Met	1.77	0.94
Phenylalanine	Phe	2.88	2.10
Threonine	Thr	2.65	3.01
Tryptophan	Trp	0.22	ND
Valine	Val	3.25	4.14
Alanine	Ala	7.66	11.40
Arginine	Arg	7.83	7.42
Aspartic acid	Asp	6.86	8.05
Cysteine	Cys	ND	0.18
Glutamic acid	Glu	11.03	9.20
Glysine	Gly	20.46	27.84
Hydroxyproline	HyP	7.73	ND
Proline	Pro	10.24	10.59
Serine	Ser	3.32	2.88
Tyrosine	Tyr	1.58	0.62
Total		23.67	101.50
Imino acids	HyP+Pro	17.97	10.59

ND: Not detected. +Chicken feet: Literature results by Liu *et al.* (2001)

Table 3: Yield, pH and swelling percentage of duck feet collagen

Content	% (except for pH)
Yield	28.37±0.58
pH	2.67±0.06
Swelling percentage	240.50±0.68

Table 3. The yield for duck feet collagen is 28.37±0.58% which is lower than yield of chicken feet collagen (30.74±0.03%) reported by Liu *et al.* (2001). Yield of collagen extracted using different acids and different soaking times range from 7.88-31.23% (Liu *et al.*, 2001). The yield is most probably depending on the proportion of fractions of different protein in the duck feet. Adjusting the pH by either lowering it to pH 4.0 or increasing it to pH 10.0 could weaken the binding ability between collagen interior molecular structures and result in swelling of collagen (Li, 1993). Other than causing higher swelling percentage, lactic acid was used as the swelling agent because it could produce higher yield of collagen with lesser loss besides possessing the greatest ability to solubilize chicken feet collagen (Liu *et al.*, 2001; Prayitno, 2007).

Gudmundsson and Hafsteinsson (1997) suggested that concentration of H^+ which is either too high or too low would result in low yield and poor gel quality. Low pH is favourable to obtain a maximum extraction rate but is detrimental to the physical properties as it produces more degradation and proliferation of lower-molecular-weight peptides (Johnston-Banks, 1990). pH value of duck feet collagen soaked with lactic acid for 24 hours is 2.67±0.06 which is slightly higher than chicken feet collagen with pH 2.54±0.02 (Liu *et al.*, 2001). Gimenez *et al.* (2005) also reported that lactic acid is an efficient disrupter for skin tissues and collagenous structures. Swelling of collagen and gel strength of lactic acid-extracted material is inversely proportional to the concentration of acid (Liu *et al.*, 2001; Gomez-Guillen and Montero, 2001). Thus, this can explain the lower swelling percentage of duck feet with 240.50±0.68% compared to chicken feet with swelling percentage of 245.85±1.63%.

Colour of duck feet collagen: Colour of duck feet collagen, commercial fish collagen, commercial cow collagen, chicken feet collagen and other fishes' gelatin is shown in Table 4. L* value of fish collagen (88.18±0.03%) is significantly higher (p<0.05) than duck feet collagen (86.98±0.02%) and cow collagen (85.82%). However, intensity of redness (0.50±001%) and yellowness (14.44±0.09%) of duck feet collagen obtained is significantly greater (p<0.05) than redness (-0.15±0.02%) and yellowness (13.87±0.06%) of fish collagen and (0.49±0.01) redness and (13.93±0.01) yellowness of cow collagen.

Compared to the results of study on chicken feet conducted by Liu *et al.* (2001), duck feet collagen (86.98±0.03%) was much lighter than chicken feet collagen (68.22±1.06%) treated with the same condition. However, intensity of redness (3.06±0.88%) and yellowness (19.27±0.17%) of chicken feet collagen is higher than duck feet collagen with redness (0.50±0.01%) and yellowness (13.87±0.06%). Apart from flavour and texture, colour plays an important quality aspect for food product. Consumer will first judge it from its appearance (colour, texture, shape) and then from other sensory attributes such as aroma or taste (Heredia, 2009). Lighter colour for duck feet collagen gives an advantage as collagen added into product does not affect the colour of the finished product. Although, information regarding the composition of duck feet that contribute to the colour of collagen is scarce, colour is suggested to be associated with the heme pigment of myoglobin and hemoglobin.

Table 4: Colour (L*, a* and b* value) of duck feet collagen, fish collagen, cow collagen, chicken feet collagen and other fishes' gelatin

Sample	L*	a*	b*	Reference
Duck feet collagen	86.98±0.03	0.50±0.01	14.22±0.09	Present study
Fish collagen	88.18±0.02	-0.15±0.02	13.87±0.06	Present study
Cow collagen	85.82±0.01	0.49±0.01	13.93±0.01	Present study
Chicken feet collagen	68.22±1.06	3.06±0.88	19.27±0.17	Liu *et al.* (2001)
Walking catfish gelatin	67.37±0.53	2.37±0.04	7.48±0.50	Jamilah *et al.* (2011)
Striped catfish gelatin	68.69±0.10	2.66±0.04	7.98±0.24	Jamilah *et al.* (2011)
Red tilapia gelatin	79.45±1.10	-0.71±0.09	5.75±0.14	Jamilah *et al.* (2011)
Shortfin scad gelatin	89.33	3.16	18.11	Cheow *et al.* (2007)
Sin croaker gelatin	91.26	2.24	13.65	Cheow *et al.* (2007)

Values are mean of each triplicated of three repeated samples with ± standard deviation

Table 5: FTIR peak locations and assignment for duck feet collagen, commercial fish collagen and commercial cow collagen

	Peak wavenumber (cm^{-1})				
Region	DFC	CFC	CCC	Assignment	Reference
Amide A	3422	3306	3306	NH stretch	Sai and Babu (2001)
Amide B	2925	2938	2959	CH_2 asymmetrical stretching	Abe and Krimm (1972)
	2854	2879	2879	CH_2 asymmetrical stretching	Abe and Krimm (1972)
Amide I	1655	1654	1656	C = O stretch/hydrogen bonding coupled with COO^-	Payne and Veis (1988)
Amide II	1593	1543	1590	NH bend coupled with CN stretch	Jackson *et al.* (1995)
	1462	1452	1451	CH_2 bend	Jackson *et al.* (1995)
Amide III	1238	1243	1242	NH bend	Jackson *et al.* (1995)

DFC = Duck feet collagen, CFC = Commercial fish collagen, CCC = Commercial cow collagen

Fourier Transform Infrared (FTIR) spectroscopy: FTIR spectrum of duck feet collagen, commercial fish collagen and commercial cow collagen are shown in Fig. 1-3, respectively. According to Abe and Krimm (1972), amide A band is related to the N-H stretching frequency. A free N-H stretching vibration falls in the range of 3400-3440 cm^{-1} (Doyle *et al.*, 1975) and will be shifted to lower frequencies, usually 3300 cm^{-1} (Li *et al.*, 2004), if there is involvement of NH group of a peptide in hydrogen bonding (Pati *et al.*, 2010). Peak of amide A for duck feet collagen was 3422 cm^{-1} which occurs within the range but peak of amide A for commercial fish collagen and commercial cow collagen were the same that is 3306 cm^{-1} which suggested existence of hydrogen bond in fish collagen and cow collagen. FTIR peak locations and assignment for duck feet collagen, commercial fish collagen and commercial cow collagen are shown in Table 5.

The amide B was observed at 2938 and 2879 cm^{-1} for commercial fish collagen and 2959 and 2879 cm^{-1} for commercial cow collagen which is higher than the 2925 and 2854 cm^{-1} for duck feet collagen. Similar to *Lates calcarifer* fish scale collagen (Sankar *et al.*, 2008), duck feet collagen fulfilled the characteristics for collagen which showed peak at 2925 cm^{-1} (2927 cm^{-1} for *Lates calcarifer* fish scale collagen) and shoulder at 2854 cm^{-1} (2861 cm^{-1} for *Lates calcarifer* fish scale collagen) which represents the CH_2-CH_3 stretching vibrations.

Surewicz and Mantsch (1988) reported that amide I band is considered to be the most useful in infrared spectroscopic analysis for secondary structure of proteins. The amide I band position of duck feet collagen, commercial fish collagen and commercial cow collagen were found at 1655, 1654 and 1656 cm^{-1} respectively, fitting well within the range of 1625-1690 cm^{-1} for general amide I band positions (Duan *et al.*, 2009). Secondary structures in the amide region which were suggested by Farrell *et al.* (2001) are: β-turns, 1700-1660 cm^{-1}; β-helix, 1654-1650 cm ; irregular structures, 1644-1640 cm^{-1} and β-sheet or extended structure, 1640-1620 cm^{-1}. There are more interaction through hydrogen bonds between C = O and adjacent chains with lower wave number is observed at amide I region (Ahmad *et al.*, 2010).

The amide II bands position of duck feet collagen detected is 1593 cm^{-1} which is within the normal absorption range of the amide II bands position 1550-1600 cm^{-1} (Duan *et al.*, 2009). Payne and Veis (1988) stated that shift of peaks to higher frequencies indicate an increase in molecular order. Jackson *et al.* (1995) reported that the amide III bands characterized the combination peaks of C-N stretching vibrations and deformation of N-H from amide linkages other than the absorptions arising from wagging vibrations from CH_2 groups from glycine backbone and proline side-chains. Similar to acid-soluble collagen prepared from carp skin, duck feet collagen also has a strong C-H stretching vibration which occurs around 2854 and 1746 cm^{-1} (Duan *et al.*, 2009).

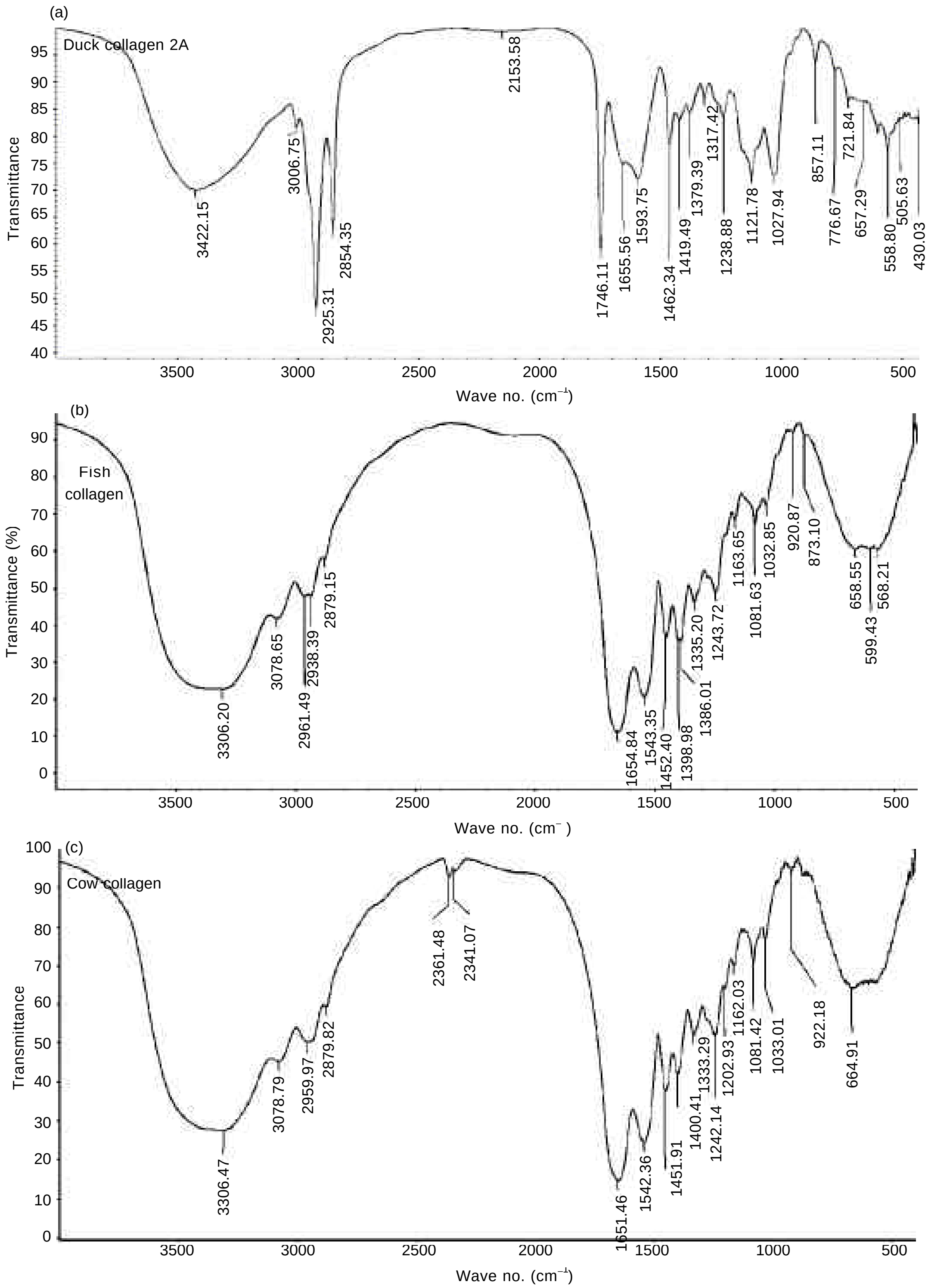

Fig. 1(a-c): Fourier Transform Infrared (FTIR) spectrum of duck feet collagen, commercial fish collagen and commercial cow collagen

Conclusion: Collagen extracted from duck feet using 5% lactic acid solution for 24 hours at 4-7°C showed chemical composition of 5.85% moisture content, 29.11% protein content, 35.43% fat content and 28.60% ash content. The amino acids content of duck feet collagen detected was 42.525mg/g and yield of duck feet collagen was 28.37%. L* value of duck feet collagen is 86.98 and duck feet collagen fulfilled the characteristics for collagen which showed peak at 2925 cm^{-1} that represents the CH_2-CH_3 stretching vibrations. Although, fat and ash content are high, modification can be done by eliminating it during the numerous washes, during the bone degreasing process, ion exchange step or alternative steps such as electro-dialysis to remove the salts. Therefore, the extraction of collagen from duck feet is another route for utilization of poultry waste.

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