Optimizing The Extraction Conditions of Gelatin Obtained from Chicken Processing By-Products

Elsanat, S. Y.(1), Korish, M.A.(2) & Ammar, A.K.(3)

(1)&(3) Food Technology Dept., Fac. of Agric. Kafrelsheikh Univ., Egypt.

(2) Food Technology Dept., Fac. of Agric. Damnhoor Univ., Egypt.

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ABSTRACT

Chicken by-products are not often processed into high-value products. One important application is the production of high quality chicken gelatin to meet the needs of markets that are not amenable to beef gelatin. So, the present study deals with the production of gelatin from chicken skin, feet and bone. The extraction process of gelatin from chicken by-products was optimized through the use of alkali (NaOH) and its effects on the protein yields and the physicochemical properties of the produced gelatin were investigated. As soaking period proceeded as yield decreased. The optimum sodium hydroxide concentration and liming period for gelatin production of chicken tissues were defined as 2.5 % for 60 hr. Yield of gelatin of chicken skin, feet and bone reached to the maximum at 60°C and 6hr extraction time. Skin, feet and bone gelatins had similar amino acid composition, with a total imino acid content of about (12.92– 16.99%). Amino acid contents of chicken tissue gelatins were different from that of commercial gelatin due to the diversity in terms of raw materials and production process.

Key word: Gelatin, feet, skin, chicken bone, alkaline conditions, amino acid composition.

INTRODUCTION

The rising interest in the valorization of industrial by-products is one of the main reasons why exploring different species and optimizing the extracting conditions of collagen and gelatin has attracted the attention of researchers in the last decade (Gómez-Guillén *et al.,* 2011). Gelatin is a hydrolyzed collagen protein (HCP). Collagen is the chief structural protein that makes up connective tissues in the body (skin, bone, cartilage, tendons, and ligaments) (Gudmundsson & Hafsteinsson, 1997). Gelatin is simply a modified form of the protein that has been broken down into smaller pieces by enzymes–which make the protein easier to incorporate into dietary products and may ease the digestion and absorption of the amino acids by the intestine. Gelatin is used in the food, pharmaceutical, and photographic industries, which take advantage of its unique properties such as reversible gel-to-sol transition of aqueous solution. Thus, worldwide overall demand for gelatin is growing every year (Marks, 1980).

Some chicken manufacture by-products i.e, skin, feet and bone are used in unprocessed form as special feed for minks and foxes and also as a feed include for other chickens after rendering. While, very little portion is being used for human consumption (Cha *et al*., 1995). However, very little studies have been carried out to investigate the feasibility of using chicken slaughter houses by-products to make food products, until recently, when the possibility of using chicken by-products instead of cow feet to make gelatin as a low-cost convenience food was probed. Inherent difference in physicochemical properties of collagen arises directly from diversification by chemical or physical modification depending on the species collagen used (Angele *et al*., 2004).

Chicken feet contain large amount of collagen, which can be easily converted into gelatin upon heating. Some are used easily processed or unprocessed, as feed for livestock (Cha, *et al.* 1995, Lim and Kim, 2001), while very little portion is used for human consumption. Since much of the chicken feet are discarded, they can easily become one of the major water pollutants if not treated in sophisticated disposal facilities. Tawfeek (1989) investigated the utilization of some by-products of poultry slaughter houses in Egypt. She found that of the three steeping pretreatments tested (i.e. acid, lime and alkali), the first proved the best on the basis of quantity and quality of gelatin (colour, odour and transparency),

while the last gave gelatin of better gel strength and viscosity. Few studies have been, however, carried out to investigate the feasibility of using chicken feet to make food products until recently, when the possibility of using chicken feet instead of cow feet to make *Jokpyun* (traditional Korean gel-type food) was probed (Jun, *et al*., 2000).

Chicken skin is chiefly used into animal meal, whereas a smaller proportion is incorporated into meat emulsions or used as a source of fat mainly for soup preparation. However, the large amount (3%) of collagen is found in chicken skin (Bonifer & Froning, 1996, Cliche *et al*., 2003) offers much greater potential for value-added products. At present, two main processes are used to extract gelatin from animal raw materials (e.g. skin and bone): an alkaline process, giving high quality products for photographic applications, and acid process, which is faster but leads to a lower quality product for food use. The lower quality is related to a lower mean molecular weight, caused by chain degradation reactions interfering with the gelatin extraction (Nicolas-Simonnot *et al*., 1997, Lim, and Kim, 2001). The conversion rate of collagen into gelatin depends on processing parameters (temperature, time and pH), the properties of the raw material and its pretreatment (Kolodziejska *et al*., 2008, Niu, *et al., 2013*). To the best of our knowledge, there are no reported studies on the production of gelatin from chicken skin and detailed physicochemical and rheological studies. As skin is a waste by-product of poultry processing, it may be possible to replace mammalian sources of gelatin with gelatin extracted from chicken skin (Sarbon *et al*. 2013).

The objective of the present study was to estimate the usefulness of different kinds of offal chicken as a source of gelatin, as well as examine the optimal alkaline extraction conditions of chicken skin, feet and bone and its effects on the yield, chemical composition and amino acid composition of obtained gelatin.

MATERIALS AND METHODS

Materials:

Frozen chicken feet, skin and bone used in the present study were obtained from local poultry plant in Alexandria city, during the summer season of 2013 and thawed overnight at 4ºC.

Methods:

Preparation of chicken feet

Fingertips of chicken feet were excised, then the feet were skinned, washed three times using tap water, cut into 2cm pieces, soaked in 1.5 L water at room temperature (25±2ºC) for 3 hr to remove blood. Then, ground by meat grinder and kept frozen at -20ºC in polyethylene bags until further processing according to the method described by Lin & Liu (2006).

Chicken skin processing

Approximately 4 kg skin were minced in Mounlix (France) laboratory meat grinder. The ground skin was then homogenized for 5 min in an Omni homogenizer (model 17105, 2-mm plate). The homogenate was heated for 1 hr at 40 ºC as measured with thermocouples in 1,000-ml beakers using a Cole-parmer thermostatically controlled bath (model 320-1106) in which the water was maintained at 45 ºC. The heated skin was centrifuged (11,400x g) for 15min, and three phases were recovered: a partially defatted protein solid phase and two liquid phase of different densities (Cliche *et al*., 2003).

Chicken bone processing:

Bones used for gelatin extraction were cleaned by scraping with a knife to eliminate some of the flesh, and then degreased by tumbling in warm water (35 ºC). The degreased bones were then demineralized using 3% HCl, at ambient temperature (20 – 25 ºC) for 3 days. Leached bones (ossein) were washed with water until pH of wash water was greater than 4. Then, bones were dehydrated in an air oven overnight at 45 ºC and then ground.

Gelatin extraction

The pre-treated materials were transferred to beakers, containing 1.5, 2.0, 2.5, 3.0 and 3.5% (w/v) of Na OH and soaked for 24, 36, 48, 60 and 72 hr. under different pH values (2, 3, 4, 5, 6, 7, 8 and 9) at different temperature degrees (40, 50, 60, 70, 80 and 90ºC).

The volume of the extracts obtained at the different treatments used and the mass of the residue (Scotch) after boiling was recorded. Portions of the gelatin extracts (light liquor) were filtered through Whatman No. 1 filter paper and were used for determining the solid concentration. The light liquor concentrations were determined by evaporating duplicate 10 ml portion to a stable weight (48 hr

at 105°C) and the concentration was used to calculate percentage of gelatin extractability. The pH of the light liquors was adjusted to about 5 using 5% ammonia solution and the extracts were dehydrated in a cross-flow air at 42°C, until brittle sheets were formed. The brittle sheets were broken into small pieces and milled using a domestic coffee grinder to pass through a 1 mm sieve. Powdered gelatin was kept at room temperature for later use (Muyonga *et al*., 2004).

Measurement of viscosity

Gelatin solutions (10% (w/v)) were made by dissolving the dry powder in distilled water and heating at 60ºC. Viscosity as a function of temperature was measured using a computerized Brookfield digital viscometer (Model DV— II, Brookfield Engineering, USA) equipped with a No. 1 spindle (Model RVT) at 60 rpm starting at 40G1ºC (Kim *et al.*, 1994).

Calculation of gelatin yield

The gelatin yield was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Dry wt. gelatin}}{\text{Wet wt. raw materials}} \times 100$$

Chemical analysis:

Analysis of chicken skin, feet, bone and their gelatins

Samples were subjected to chemical analysis. Moisture was determined by drying in an air oven at 105˚C to a constant weight, crude protein, by using the Micro-Kjeldahl method to determine the total nitrogen and multiply its value by the factor of 5.4, ether extract, in a Soxhlet apparatus using petroleum ether (40 - 60ºC) as a solvent and ash content by ashing in an electric muffle at 550˚C until constant weight. The above experiments were determined according to the standard methods of the AOAC (2000). All determinations were calculated on dry weight basis.

Rheological properties

A dynamic temperature sweep rheological test was used to determine the gelation and the melting temperature of the gelation samples. The stress and frequency used were 0.1 Pa and 1 rad/s, respectively. For gelation, the sample was initially maintained at a temperature of 40°C for 10 min to allow for equilibration. Gelation samples were cooled on a Peltier plate from 40°C to 10°C and heated back to 40°C both at a scanning rate of 2°C /min. The gelation temperature was taken to be the temperature at which the elastic modulus began to dramatically increase in value. The temperature at which the *G'*/*G''* cross over occurred during cooling is close to the sol-gel transition or the gel formation point (Gudmundsson, 2002). The test for determining melting temperature immediately followed after the gelation test. After the sample reached 10°C, the temperature was raised back to 40°C. Melting occurred when the elastic modulus (*G'*) began to decrease and loss modulus (*G''*) began to increase in value. Changes in the elastic or storage modulus (*G'*) (describing the amount of energy that is stored elastically in the structure and the viscous or loss modulus) and loss modulus (*G''*) (indicating the amount of energy loss or the viscous response) were determined as a function of temperature and were recorded.

Amino acid composition

Amino acid analysis was conducted using the Pico. Tag method (Bildlingmeyer *et al*., 1984). This method involves derivatisation of amino acids using phenylisothiocyanate (PITC) and determination of the phenylthiocarbamyl (PTC) amino acids using reversed phase HPLC. Dry gelatin (10 – 20mg) was mixed with 6 M HCl (1 ml) containing 1% phenol (v/v). The mixture was evacuated, blown with N_2 and vacuum sealed before hydrolysis at 110°C for 24 hr. After hydrolysis, the samples were cooled and made up to 5 ml with deionized water. A portion (25µl) was then dried and derivatised. Derivatisation involved addition of 10 µl of a mixture of methanol, water and trimethylamine (2:2:1, v/v/v), mixing and then drying for 5 min. This was followed by addition of 20 µl of a mixture of methanol, water, trimethylamine and phenylisothiocyanate (7: 1: 1: 1, v/v/v/v). The sample was washed with deionized water for 20 min at room temperature (20 – 25 °C), dried under vacuum and then dissolved in 200 µl of pH 7.4 phosphate buffer and filtered with a 0.45 µm filter. Portions (20 µl) of the filtered samples were injected using an automatic loader (WISP™) (Millipore Corp., Milford, MA,USA) into the Pico. Tag column, part no. 88131 (3.9 mm × 13 cm) (Millipore Corp, Milford, MA, USA), for amino acid analysis.

Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using general linear model

(GLM) of SX according to Steel & Torrie (1980) to determine the significant differences ($P < 0.01$). Mean comparisons were performed using Tukey's multiple range test. Response surface methodology (RSM) was used to determine optimum concentrations of sodium hydroxide, soaking time, pH and temperature for the liming process of chicken skin, feet and bone to produce gelatin. The experiment was repeated three times.

RESULTS AND DISCUSSION

Gross chemical composition of raw chicken by-products

Table (1) shows the proximate composition of the chicken skin, feet and bone. The protein content of the chicken skin, feet and bone was found to be 36.5, 43.0 and 29.5, respectively. The protein content of the collagenous material represents the maximum possible yield of gelatin expected from them. This was higher for feet than for skin and bone. The bone generally contained higher ash and lower moisture than the skin and feet. Chicken skin was found to contain more ether extract than feet and bone, probably because the chicken accumulates subcutaneous fat under skin. These results are near from those obtained by Nicolas-Simonnont *et al*. (1997), Sarbon *et al*. (2013).

Table 1: Proximate composition of chicken skin, feet and bone (% on dry weight basis)

Component (%)	Skin	Feet	Bone
Moisture	32.0[a]	20.5[b]	12.5[c]
Dry matter	68.0[c]	79.5[b]	87.5[a]
Crude protein	36.5[c]	43.0[a]	29.5[b]
Ether extract	49.5[a]	16.5[b]	2.4[c]
Ash content	11.5[c]	40.5[b]	68.1[a]

Means within a row not sharing superscript are significantly different ($P < 0.01$, Tukey test) (N=3).

Effect of alkaline concentration on extractability of chicken skin, feet and bone gelatins (yield%)

The effect of the calcium hydroxide concentration and liming period on the yield of gelatin are shown in Tables (2) and (3). Data show that both the calcium hydroxide concentration and liming period significantly affected the gelatin yield. The chicken feet had the highest amount of gelatin comparing with that obtained from skin or bone.

Table 2: Effect of alkaline concentration on the extractability of chicken skin, feet and bone gelatin (Yield %, on dry weight basis)

Alkaline concentration %(w/v)	Skin[(B)]	Feet[(A)]	Bone[(C)]
1.5	13.06[d]	10.77[d]	5.36[d]
2.0	13.89[c]	15.89[c]	6.08[c]
2.5	19.83[a]	24.65[a]	9.63[a]
3.0	16.70[b]	23.80[b]	7.84[b]
3.5	9.93[e]	7.05[e]	3.76[e]

Extraction conditions: pH 7.0, 60 ºC, soaking time in alkaline 48 hr and extraction time 6 hr.
(A), (B) and (C): comparison of means of yield % by type of tissue.
a, b, c, d and e: comparison of means of yield % by alkaline concentration%(w/v).
Means within a column not sharing the same superscript are significantly different ($P < 0.01$, Tukey test) (N=3).

Table 3: Effect of soaking period in alkaline on extractability of chicken skin, feet and bone gelatin (Yield %, on dry weight basis)

Soaking period (hr)	Skin[(B)]	Feet[(A)]	Bone[(C)]
24	12.79[e]	15.93[e]	1.83[e]
36	14.85[d]	16.19[d]	5.51[d]
48	17.77[c]	21.80[c]	6.05[c]
60	19.83[a]	24.65[a]	9.63[a]
72	19.05[b]	23.06[b]	7.99[b]

Extraction conditions: pH 7.0, 60ºC, 2.5% alkaline and extraction time 6hr.
(A), (B) and (C): comparison of means of yield % by type of tissue.
a, b, c, d and e: comparison of means of yield % by soaking time (hour).
Means within a column not sharing the same superscript are significantly different ($P < 0.01$, Tukey test) (N=3).

The yield of gelatin increased with the higher concentration of calcium hydroxide and longer liming period. This result confirmed the study of Lim and Kim (2001), who reported that the yield of chicken feet gelatin increased with longer liming period up to iso-electric point. However, Kim *et al*. (1994) reported that the yield of fish gelatin decreased with increasing the calcium hydroxide concentration over 1.5% and liming period over 5 days. Their explanation might be due to the softer fish skin than the cattle hide and also to the degree of decreased freshness with the increased liming period. Yield of gelatin from chicken skin, feet and bone were (9.93

– 19.83%), (7.05 – 24.65%) and (3.76 – 9.63%), respectively. The lower yield may be due to the loss of extracted collagen through leaching during the series of washing steps or due to the incomplete hydrolysis of the collagen (Sarbon *et al*., 2013).

Effect of pH value on extractability of chicken skin, feet and bone gelatins (yield %)

One of the most important parameters to be considered for gelatin extraction is the extracellular pH. Table (4) presents the results achieved for the gelatin extraction (yield %) at different pH (2-9). The results show that, gelatin yield (%) was enhanced by elevating the pH value. The lowest values (17.08, 14.04 and 6.02%) were recorded for chicken skin, feet and bone respectively, at pH 9. On the other hand, the highest values were recorded at pH 4.0 and 3.0, respectively. This might be due to the hydrolysis of cross-linkage in collagen and other proteins by excessive acid treatment following alkali treatment. The data revealed a significant effect ($P<0.01$) of this factor on the production of gelatin by pH. Similar results were obtained by Lim and Kim (2001), who found that the yield of chicken feet gelatin increased when higher concentrations of hydrochloric acid and citric acid were used to neutralize the chicken feet before extraction. The transmittance of gelatin was depended on the final pH of the product rather than the concentrations of the acid solutions used in the neutralizing process.

Table 4: Effect of pH value on extractability of chicken skin, feet and bone gelatins (Yield %, on dry weight basis)

pH	Skin(B)	Feet(A)	Bone(C)
2	17.89^{d}	18.90^{d}	9.97^{d}
3	22.31^{b}	25.23^{b}	13.31^{b}
4	24.03^{a}	27.13^{a}	14.96^{a}
5	20.87^{c}	24.67^{c}	14.10^{ab}
6	19.79^{cd}	24.18^{c}	11.18^{c}
7	19.83^{c}	24.65^{c}	9.63^{d}
8	18.32^{d}	19.28^{d}	8.02^{e}
9	17.08^{e}	14.04^{e}	6.02^{f}

Extraction conditions: soaking time in alkaline 60h, 60 ºC, 2.5% alkaline and extraction time 6h.
(A), (B) and (C): comparison of means of yield % by type of tissue.
a, b, c, d and e: comparison of means of yield % by pH.
Means within a column not sharing the same superscript are significantly different ($P <0.01$, Tukey test) (N=3).

Effect of temperature on the extractability of chicken skin, feet and bone gelatin (yield %) and viscosity (MPas)

Temperatures have significant effect on gelatin extractability. Table (5) shows the effect of temperature on gelatin production at different temperatures (40–90˚C). The data showed that gelatin yield seemed to be stimulated by elevating the temperature from 40 to 60˚C enhanced the yield %, of skin and feet gelatin.

Notwithstanding, the lowest values (15.00, 18.14 and 35.70%) were recorded for a gelatin yield obtained from chicken skin, feet and bone, respectively at 90˚C. Table (5) also shows differences among skin, feet and bone. It can be noted that, feet had the highest gelatin yield than skin and bone. This is consistent with reports that the three types of tissue vary in type's quantities of crosslinks

Table 5: Effect of temperature on extractability of chicken skin, feet and bone gelatins (Yield %, on dry weight basis) and viscosity (MPas).

Temperature (ºC)	Skin(B)		Feet(A)		Bone(C)	
	Yield (%)	Viscosity	Yield (%)	Viscosity	Yield (%)	Viscosity
40	16.22^{d}	70.45^{a}	19.33^{d}	79.20^{a}	81.90^{a}	10.78^{d}
50	17.86^{c}	69.45^{b}	21.95^{c}	78.45^{b}	80.55^{a}	12.17^{c}
60	24.03^{a}	32.15^{c}	27.13^{a}	34.65^{c}	36.80^{a}	15.51^{a}
70	23.16^{ab}	31.65^{c}	26.78^{a}	34.95^{c}	36.55^{a}	14.96^{a}
80	21.31^{b}	31.05^{c}	24.18^{b}	32.70^{d}	35.85^{a}	13.22^{b}
90	15.00^{e}	31.55^{c}	18.14^{e}	32.50^{d}	35.70^{a}	10.13^{e}

Extraction conditions: soaking time in alkaline 60 h, pH 4, 2.5% alkaline and extraction time 6 h.
(A), (B) and (C): comparison of means of yield % by type of tissue.
a, b, c, d and e: comparison of means of yield % by temperature (ºC).
Means within a column not sharing the same superscript are significantly different ($P <0.01$, Tukey test) (N=3).

(Sims *et al*., 2000, Muonga *et al*., 2004, Sarbon *et al*., 2013). The data in the present study suggest that chicken feet collagen contain markedly lower amount of stable crosslinks than skin and bone collagen. These results agree with those reported by Sims *et al*. (2000), Muyonga *et al*. (2004) and Sarbon *et al*. (2013) who reported markedly higher extractability of gelatin at low temperature for younger cattle hides and pigskin. Also, in the case of Nile perch, however, extrability of gelatin was high (66.3% at 50˚C) from adult fish skin.

Table (7): Proximate composition of chicken skin, feet, bone and bovine gelatin (% on dry weight basis).

Component (%)	Skin	Feet	Bone	Bovine (Sarbon *et al*., 2013)
Moisture	11.20^{ab}	11.47^{a}	10.61^{c}	9.68^{d}
Dry matter	88.82^{c}	88.53^{c}	89.21^{ab}	90.32^{a}
Crude protein (N×5.4)	81.48^{ab}	81.18^{b}	80.00^{c}	81.75^{a}
Ether extract	0.34^{b}	0.35^{a}	0.31^{c}	-
Ash	1.31^{c}	2.50^{b}	4.52^{a}	1.06^{d}

Means within a column not sharing the same superscript are significantly different (P <0.01, Tukey test) (N=3).
a, b, c and d: comparison of means of yield % by type of tissue.

Effect of heating period on extractability of chicken skin, feet and bone gelatins (yield %)

The values of gelatin yield as a function of heating period are shown in Table (6). The gelatin yield was markedly affected by the heating period. The results showed that the yield was positively influenced and increased markedly with longer heating period up to certain level and then decreased. This trend of results is similar to that obtained by Jun *et al*., (2000) and Sarbon *et al*. (2013).

Table (6): Effect of extraction period on extractability of chicken skin, feet and bone gelatins (Yield %, on dry weight basis).

Heating period (h)	Skin$^{(B)}$	Feet$^{(A)}$	Bone$^{(C)}$
3	16.50^{e}	17.75^{c}	7.70^{e}
4	18.63^{c}	21.31^{b}	9.97^{d}
5	22.32^{b}	26.35^{a}	13.65^{b}
6	24.03^{a}	27.13^{a}	14.96^{a}
7	22.17^{b}	26.73^{a}	14.47^{a}
8	17.14^{d}	21.29^{b}	11.67^{c}

Extraction conditions: soaking time in alkaline 60 h, pH 4, 2.5% alkaline and 60 ºC.
(A), (B) and (C): comparison of means of yield % by type of tissue.
a, b, c, d and e: comparison of means of yield % by heating time (h).
Means within a column not sharing the same superscript are significantly different (P <0.01, Tukey test) (N=3).

Proximate composition of gelatin derived from chicken skin, feet and bone comparing with bovine gelatin.

The proximate composition of gelatin obtained was found to vary with type of tissue used as raw material (Table 7). Generally, the gelatin samples extracted were almost free of fat (<0.5%). This showed that the processes used had eliminated fat as desired. The skin gelatin was low in ash, comparing with feet and bone gelatin. The latters had much higher ash content being, 2.50 and 4.52%, respectively, indicating that the leaching process was inadequate.

The similarities in proximate composition of gelatin obtained from chicken wastes and bovine gelatin indicate that chicken waste my be used as a potential alternative source for gelatin. Manufacture of chicken bone gelatin may therefore, require an ion exchange step to remove the excess of salts or improve the leaching process, which can be achieved by application of counter-current process. This result is in accordance with the results of Muyonga *et al*. (2004) and Sarbon *et al*. (2013).

Rheological properties of gelatin gels

The gelling and melting temperatures and the dynamic viscoelastic profile of chicken and bovine gelatin at a concentration of 6.67% (w/v) are presented in Table (8). The maximum values of elastic (G') and loss (G'') modulus of chicken gelatin (8275, 6640 Pa, respectively) were significantly higher than that for bovine gelatin (4330, 4122 Pa, respectively) ($P < 0.05$). Although the melting temperature of chicken gelatin (33.57°C) was significantly higher ($P < 0.05$) than that of bovine gelatin (31.55°C), there was no significant difference ($P < 0.05$) in the gelling temperature (24°C) for both chicken and bovine gelatin. The gelling temperature is the temperature at which the G′/G′′ crossover occurred on cooling and is close to the solegel transition (Gudmundsson, 2002).

Table 8: Rheological properties of chicken skin, feet, bone and bovine gelatin (6.67%) including gelling and melting temperature, elastic (G′) and loss (G′′) modulus values after heating to 40°C and cooling to 10°C.

Gelatin 6.67 (%)	Gelling temp. (°C)	Melting temp. (°C)	Maximum value after cooling	
			G′ [Pa]	G′′ [Pa]
Chicken skin	24.95[a]	33.70[a]	8275[a]	6640[ab]
Chicken feet	22.35[d]	31.35[c]	8146[c]	6632[bc]
Chicken bone	23.45[c]	32.45[b]	8221[b]	6621[c]
Bovine (Sarbon *et al.*, 2013)	24.43[b]	31.55[c]	4330[d]	4122[d]

Means within a column not sharing superscript are significantly different (P <0.01, Tukey test) (N=3).
a, b, c and d: comparison of means of yield % by type of tissue.

Table (8) compares the dynamic viscoelastic profile of chicken skin and bovine gelatin during both cooling from 40 to 10°C and heating from 10 to 40°C at constant rate of 2°C/min. During cooling, G' values increased sharply due to an increase in the amount of energy that is elastically stored, which indicates rapid formation of junction zones and a strong reinforcement of the gel network. Chicken gelatin showed higher elastic modulus (G') values at low temperature indicative of enhanced ability to refold into a triple helix (Gómez-Guillén *et al*., 2002). The higher elastic modulus (G') of chicken gelatin showed that a higher thermal transition was required as compared to the bovine gelatin both during cooling and heating, which indicates that it was more heat stable. Generally, high G' values and thermo-stability are typical of mammalian gelatin (Gilsenan & Ross-Murphy, 2000) and are mainly related to imino acid composition, with hydroxyproline playing a unique role in stabilizing the triple helix. Gómez-Guillén *et al*. (2002) correlated the thermal stability of gelatin to the number and stability of pro-rich regions in collagen and gelatin molecules, which are high in mammalian species and fresh warm water fish as compared with cold water fish.

Amino acid composition

The amino acid composition of chicken skin, feet, bone and bovine skin gelatin are shown in Table (9). The skin, feet and bone collagens have similar amino acid composition. The observed differ-

Table (9): Amino acid composition (%) of chicken skin, feet, bone and bovine gelatin

Amino acids	Skin	Feet	Bone	Bovine (Sarbon *et al.* 2013)
Indispensable amino acids:				
Thereonine	6.63	6.82	6.01	0.82
Valine	1.38	1.27	1.45	2.07
Methionine	1.62	2.26	1.11	0.22
Isoleucine	1.28	0.93	1.29	1.01
Leucine	2.1	1.38	1.94	1.89
Phenylalanine	1.2	1.50	0.87	1.60
Tyrosine	6.63	6.82	6.01	1.16
Lysine	3.28	2.57	2.72	4.86
Histidine	1.59	1.53	1.62	-
Arginine	5.61	5.17	5.09	5.09
Total indispensable amino acids	31.34	30.25	28.11	18.72
Dispensable amino acids				
Asparatic acid	10.21	10.98	9.41	3.29
Serine	1.99	1.75	1.63	10.67
Glutamic acid	15.10	16.00	14.36	5.43
Proline	9.71	10.36	9.31	12.66
Hydroxy Proline	3.21	6.63	6.27	10.67
Alanine	9.73	9.43	8.00	8.41
Glycine	18.71	14.60	22.91	30.15
Total dispensable amino acids	**68.66**	**69.25**	**71.89**	**81.28**
Imino acid (Pro+Hyp)/total amino acids%	**12.92**	**16.99**	**15.58**	**23.33**

ences in the functional properties of gelatins are not due to the differences in the amino acid content. The amino acid composition of different chicken tissue types, was, however, different from those reported for other gelatins from different animal sources. Imino acid (proline + hydroxyl proline) content of chicken gelatins (12.92 – 16.99%) was lower than that reported for Nile perch gelatin (Muyonga *et al*., 2004). The same author mentioned that imino acid content is a key determinant of the melting and setting temperatures of the gelatins. Also, Jang *et al*. (2000) found that proline content of chicken feet under alkaline condition was 2.58%. On the other hand, Nicolas- Simonnot *et al*. (1997) found that the imino acid content of hard bone was 30%.

The amino acid content of gelatins has a strong influence on their functional properties (Gilsenan & Ross-Murphy, 2000). The content of imino acids (proline and hydroxyproline) are of particular importance regarding both gelatin gel strength and melting point. Due to the rigidity of their R groups the imino acid provide rigidity to triple helix structures both in intact collagen and gelatin gels (Haug *et al*., 2004, Arnesen & Gildberg, 2007, Sarbon *et al*., 2013).

CONCLUSION

Chicken gelatin extracted from skin, feet and bone waste by-products can provide an alternative source of gelatin as it shows similar chemical composition to bovine gelatin and better physicochemical properties as compared with reported fish gelatins. Both formed stable structures on cooling. Chicken gelatin revealed greater increases in the G' and G'' values with increase in concentration, as compared to bovine gelatin. The strength of gelatin gels, measured as a function of frequency sweeps showed that G' values for chicken gelatin were higher than those of bovine gelatin at all concentrations tested and stable in the frequency range tested.

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الظروف المثلى لاستخلاص الجيلاتين من النواتج الثانوية للدواجن

سمير يوسف السناط[١]، محمد عبد الرؤوف قريش[٢]، وأمين كمال عمار[٣]

(١)، (٣) قسم تكنولوجيا الأغذية – كلية الزراعة – جامعة كفر الشيخ – مصر

(٢) قسم تكنولوجيا الأغذية – كلية الزراعة – جامعة دمنهور – مصر.

عادة لا يتم تحويل المنتجات الثانوية لصناعة الدواجن إلى منتجات ذات قيمة. لذا هدفت هذه الدراسة إلى محاولة إنتاج جيلاتين عالي الجودة من أرجل وجلد وعظام الدجاج لتلبية احتياجات الأسواق وتعويض النقص في الجيلاتين البقري. تم دراسة تأثير استخدام تركيزات مختلفة من هيدروكسيد الصوديوم على إنتاج الجيلاتين من جلد وأرجل وعظام الدجاج. وكان أفضل تركيز من القلوي ٢,٥٪ ، النقع لمدة ٦٠ ساعة باستخدام درجة حرارة ٦٠°م وفترة استخلاص ٦ ساعات، حيث أعطت أعلى إنتاج من الجيلاتين وأفضل صفات لبروتين الجيلاتين وخصائصه الطبيعية والكيميائية. وقد أدت زيادة فترة النقع عن ذلك إلى انخفاض العائد من الجيلاتين. كما أوضحت النتائج تشابه محتوى الأحماض الأمينية في كل من جيلاتين الجلد والأرجل والعظام وبلغت نسبة الأحماض الأمينية ١٢,٩٢ إلى ١٦,٩٩٪ بالنسبة لمجموع الأحماض الأمينية. من ناحية أخرى فقط كان محتوى الأحماض الأمينية للجيلاتين المستخلص من أنسجة الدجاج مختلفا عن الجيلاتين التجاري وذلك لاختلاف المواد الخام وظروف عملية الإنتاج.