Optimum Condition of Extracting Collagen from Chicken Feet and its Characetristics

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ABSTRACT : The objective of this research was to evaluate alternative treatments for the best extraction condition for collagen from chicken feet. Various properties such as chemical composition, amino acid, pH, swelling percentage, yield and pure collagen, collagen loss, color (Hunter L, a and b) and electrophoresis of collagen from chicken feet treated by 5% acids (acetic acid, citric acid, hydrochloric acid and lactic acid) and soaking times (12, 24, 36 and 48 h) were evaluated. The crude protein, fat, ash and moisture contents of chicken feet was 17.42, 12.04, 5.98 and 62.05%, respectively. Amino acid composition of collagen from chicken feet indicated that the protein of collagen was markedly hydrolized by the hydrochloric acid treatment. The result of electrophoresis also supported this phenomenon. Both the swelling percentage of lactic acid and citric acid treatments were significantly higher than that of acetic acid and HCl treatment. The pH of the acid treatments ranged from 2.43-3.62. According to the result of yield, pure collagen and loss of collagen, the best condition of extracting collagen from chicken feet was soaked in 5% lactic acid for 36 h. However, a brighter yellow color of collagen from all treatments was observed with a longer soaking time. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 : 1638-1644)*

Key Words : Chicken Feet, Collagen, Swelling Percentage

INTRODUCTION

Collagen is the most abundant and ubiquitous protein in the body and which accounts for about thirty percent of the total human body protein. There exist at least fourteen genetically distinct types of collagen. The most familiar, type I, consists of three polypeptide chains. Two chains identical and are call α1; the third being call α2. Type I collagen is the major portion of the collagen of both soft (skin, tendon) and hard (bone and dentine) connective tissue. Type II collagen is the major collagen of cartilage and is composed of three α1 chains. Type III collagen is composed of three α1 (III) chains and is found in blood vessel, wounds and certain tumors. Generally, collagen has been extracted from mammary animals (cattle and pig skin) for food, cosmetics and medical products. Ichie et al. (1999) reported that a low allergenic gelatin could be produced from chicken cartilage by acid processing. Collagen types I, II, III and V were isolated and identified from mechanically deboned chicken meat (MDCM) of chicken neck parts, and type I was the major component (Tanaka et al., 1996). Todbunter et al. (1994) stated that collagen type I and II were purified from equine skin and flexor tendon as well as articular cartilage. Some studies indicated that type II collagen showed reduced arthitic response when used as a treatment on rats or mice (Phadke et al., 1984; Zhang et al., 1990; Yoshino, 1996; Taguchi et al., 1999). Trentham et al. (1993) also observed a decreased incidence of swollen joint and tender joints in subjects fed chicken collagen type II for 60 patients with severe active rheumatiod arthritis for 3 month. From this information, collagen type II from animal can be used as an effective oral medicine to cure patients with severe active rheumatiod arthritis. Poultry feet are abundant in collagen and also have been extracted as medical material such as collagen film, and collagen powder for wound exudate control (Pachence, 1992; Li, 1993).

Recently, consumers had rejected some foods and cosmetics that were prepared from beef collagen or gelatin due to the fear of bovine spongiform encephalopathy. Thus, it is desirable to seek an alternative source of collagen and gelatin from the other animal species rather than from cattle. Chicken feet maybe a good collagen source and could be used to replace beef collagen. The objective of this research was to evaluate alternative treatments for the best condition of extracting collagen from chicken feet. Some properties such as chemical composition, amino acids, pH, color, collagen content, yield, collagen loss and collagen type identification were determined in this research.

MATERIALS AND METHODS

Preparation of chicken feet collagen

The frozen chicken feet (broiler) were obtained from a local poultry meat plant in the central area of Taiwan and stored at -20°C for this study. Chicken feet were thawed at 4°C for 24 h, and then ground utilizing a 10 mm and 0.4 mm plate in sequence. The isolation of collagen from chicken feet paste was performed by the following procedures. The ground chicken feet were mixed with 5% of different acid solutions (acetic acid, citric acid,

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hydrochloric acid and lactic acid) by w/v (chicken feet/ solution=1/8) and soaked for different times (12, 24, 36 and 48 h) at 4-7°C. At the end of soaking, differently-treated chicken feet suspended solutions were homogenized by a blender (31BL91, Warning Blender, USA) with 10,000 rpm for 5 min (45 sec work and 15 sec rest), and then filtered by a double gauze to discard bone residues. These suspended solutions were neutralized to pH 7 with 0.1 N NaOH. The neutral solutions were centrifuged with a high speed centrifuge (SCR208, Hitachi, Japan) at 8,000 g for 15 min at 10°C. The supernatant was discarded and the precipitate was lyophilized by a freezer dryer (FDU-540, EYELA, Japan) to obtain a dry collagen.

Analysis of chemical composition and amino acid

The chemical contents (crude protein, crude fat, moisture and ash) of chicken feet were analyzed by AOAC (1995). Hydrolysis and preparation for amino acid analysis of chicken feet and chicken feet collagens were done by the procedure outlined by Burgos et al. (1974). The samples were hydrolyzed in 6 N HCl at 110°C for 24 h and measured by an amino acid auto analyzer (Model 6300, Beckman, U.S.A.). The amino acid content was expressed as mole (%).

Analysis of pH and swelling percentage

The pH of crude collagen samples was measured at the end of the different soaking times, and measured utilizing the procedure of Ockerman (1984). Swelling percentage was calculated utilizing 【the weight of the super-solid from filtered product at the end of different soaking times (12, 24, 36 and 48 h) by a stainless filter / the weight of chicken ground feet before soaking】 × 100.

Analysis of yield, pure collagen and collagen loss

The yield (%) of crude collagen was calculated by the following formula - 【the weight of frozen dry crude collagen from different treatments / the weight of ground chicken feet】 × 100. The collagen content of crude collagen samples was determined by the procedure of Reddy and Enwemeka (1996). The collagen loss (%) was expressed by 【9.07 (collagen of chicken feet) subtracted from the collagen of crude collagen samples / 9.07】 × 100.

Color determination

The color of crude collagen from chicken feet was measured by a colorimeter (NR-3000, Handy, Japan), and Hunter L, a and b values were used to indicate lightness, redness and yellowness of sample in this study.

Analysis of SDS-PAGE

The sodium dodecyl sulfate (SDS) polyacrylamide gel (containing 7.5% acrylamide) was utilized to distinguish different types of collagen from chicken feet (O'Driscoll et al., 1985). A volume of 10-20 μL of sample buffer, containing 200 μg of sample was loaded into slab gel. A constant current of 15 mA was used before the dye front reached the separating gel and then the current was increased to 25 mA and maintained until the electrophoresis was completed (approximately for 4 h). The gels were stained for 1 h in a solution of 0.25% Coomassie brilliant bule-R-250 in methanol:acetic acid:water (5:1:10) and destained in a solution of methanol:acetic acid:water (2:3:35).

Statistics analysis

All data of this experiment were analyzed by a GLM program and Duncan's new multiple range test found in the SAS system (1989).

RESULTS AND DISCUSSION

The chemical composition and amino acid contents

The chemical composition of chicken feet is shown in table 1. Data indicated that chicken feet contained a lower protein content (17.42%) than that of calf skin (30-35%) and pig skin (31%) (Balian and Bowes, 1977; Chow, 1981; Bailey and Light, 1989). The collagen amount of chicken feet was 9.07% which is also lower than that of skin, tendon, bone, stomach and lung of mammalian animals (15-95%), but higher than other organs such as kidney and liver (Balian and Bowes, 1977; Bailey and Light, 1989). The moisture content was 62.05% which is similar to that of calf and pig skin (60-73%) but had a higher ash content (5.98%) than that of pig skin (0.35%) (Chow, 1981; Wang, 1994). The higher ash content of chicken feet could be caused by a high level of bone residue. The crude fat content of chicken feet was 12.04% and ranged between pig skin (20-25%) and calf skin (1-10%) (Balian and Bowes, 1977; Chow, 1981).

The amino acid composition of chicken feet and collagens from chicken feet soaked with 5% of various acids (acetic acid, citric acid, hydrochloric acid and lactic acid) for 36 h is shown in table 2. Asghar and Henrickson (1982) stated that twenty or twenty-one different amino acids are known to be present in different collagen types. In this experiment, 21 amino acids were found to be contained in chicken feet. It can be found that 30% of the total amino

Table 1. The chemical composition of chicken feet[1]

Content	%
Moisture	62.05±0.60
Crude fat	12.04±0.44
Crude protein	17.42±0.73
Collagen	9.07±0.18
Ash	5.98±0.37

n=6.

[1] Values are mean ± standard deviation.

Table 2. Amino acid composition of chicken feet and chicken feet collagen from 5% different acids and soaked for 36 h

Amino acid Mole (%)	Chicken feet	Chicken feet collagen			
		Acetic acid	Citric acid	Hydrochloric acid	Lactic acid
ASP	8.65	9.10	9.84	16.22	8.17
THR	3.27	3.27	3.78	6.74	3.05
SER	3.09	3.23	3.37	6.51	2.92
GLU	10.04	10.11	11.29	19.09	9.34
PRO	11.72	12.08	13.43	10.79	10.75
GLY	30.17	32.66	36.05	23.79	28.25
ALA	12.67	12.96	14.26	15.61	11.57
CYC	0.29	0.19	0.30	0.44	0.18
VAL	4.95	4.72	5.52	10.18	4.20
MET	1.15	1.21	1.27	2.29	0.95
ILE	2.65	2.35	2.73	7.09	2.30
LEU	5.17	4.64	5.46	13.25	4.41
TYR	0.90	0.79	1.41	2.25	0.63
PHE	1.84	2.12	2.57	5.04	2.13
HIS	1.35	0.99	1.42	3.11	1.00
LYS	4.70	4.19	5.05	8.93	4.11
ARG	8.14	8.50	9.02	9.99	7.53

acid residue consist of glycine, approximately 11.7% of proline and 10-12.7% of alanine and glutamic acid. Relatively small amounts of the other 14 amino acids account for the remaining proportion of the residue. The content of lysine, histidine, phenylalanine, isoleucine, tyrosine and methione is approximately 2% or less for each. The result of this research was similar to the distribution of chicken tendon and pig skin reported by Eastoe and Leach (1977). A higher percentage about 28.2, 32.6 and 36% of glycine were found in collagen from chicken feet individually soaked with 5% lactic acid, acetic acid and citric acid respectively. A very low percentage about 23.7% of glycine was found in collagen from chicken feet soaked with hydrochloric acid when compared to the other treatments. At the same time, the other 20 amino acid contents of collagen from chicken feet soaked with hydrochloric acid for 36 h were higher than those of the other treatments. These results suggest that amino acid of chicken feet collagen can be seriously broken down by strong acid and resulted in the decrease of the major amino acid contents. However, the amino acid composition of chicken collagen soaked with acetic acid, citric acid and lactic acid is similar to that of collagen from pig skin and cow skin (Asghar and Henrickson, 1982).

The swelling percentage and pH

Li (1993) stated that collagen can be swelled due to weakening of binding ability between collagen interior molecular structure when pH is lowered to 4 or raised up to 10. In this experiment, four 5% acid solutions (acetic acid, citric acid, lactic acid and hydrochloric acid) were used as swelling agent for chicken feet and the results of swelling percentage are showed in table 3. The average swelling percentage of the samples from different soaking times (12, 24, 36 and 48 h) with citric acid (247%) and lactic acid (246.5%)was significantly higher ($p<0.05$) than that of the samples soaked with acetic acid (227.8%) and hydrochloric acid (129.91%). Ashar and Henrickson (1982) also stated that acetic acid produces 50% more swelling and a greater degree of peptidization of collagen than HCl does at pH 2.0. As the concentration of acid increases the swelling of

Table 3. Swelling percentage[1] of chicken feet treated with 5% different acids and soaking times

Time (h)	Acetic acid	Citric acid	Hydrochloric acid	Lactic acid
12	218.55 ± 10.67^{by}	232.50 ± 8.06^{by}	134.05 ± 1.91^{az}	239.60 ± 5.23^{by}
24	228.80 ± 3.54^{aby}	243.35 ± 17.89^{abx}	123.65 ± 3.18^{az}	245.85 ± 1.63^{aby}
36	222.90 ± 17.68^{aby}	247.95 ± 19.45^{abx}	129.20 ± 6.79^{az}	248.65 ± 2.42^{abx}
48	230.95 ± 9.26^{ay}	264.15 ± 8.55^{ax}	132.75 ± 0.21^{az}	251.75 ± 4.31^{ax}
Average	227.80 ± 4.21^{y}	247.00 ± 11.45^{x}	129.91 ± 1.58^{z}	246.50 ± 2.78^{x}

n=10.

[1] Values are mean ± standard deviation.

[a,b,c]: Means within the same column without the same superscript are significantly different ($p<0.05$).

[x,y,z]: Means within the same row without the same superscript are significantly different ($p<0.05$).

Table 4. The pH[1] of collagen from chicken feet treated with 5% different acids and soaking times

Time (h)	Acetic acid	Citric acid	Hydrochloric acid	Lactic acid
12	3.44 ± 0.01^{bx}	2.43 ± 0.04^{cz}	N.d.	2.72 ± 0.06^{ay}
24	3.47 ± 0.02^{bx}	2.50 ± 0.08^{by}	N.d.	2.54 ± 0.02^{by}
36	3.62 ± 0.02^{ax}	2.63 ± 0.09^{ax}	N.d.	2.65 ± 0.04^{bx}
48	3.54 ± 0.03^{bx}	2.57 ± 0.02^{abyz}	N.d.	2.71 ± 0.01^{az}

n=10.

[1] Values are mean ± standard deviation.

[a,b,c]: Means within the same column without the same superscript are significantly different ($p<0.05$).

[x,y,z]: Means within the same row without the same superscript are significantly different ($p<0.05$).

N.d.: not detected.

Table 5. The yield, collagen content[1], pure collagen[1], collagen loss[1] of chicken feet treated with 5% different acids and soaking times

Item	Yield(%)	Collagen (%)	Pure collagen g / 100g	Collagen loss (%)
Acetic acid				
12 h	30.69 ± 0.33^{a}	17.25 ± 0.96^{c}	5.46 ± 0.30^{c}	39.80 ± 1.25^{e}
24 h	30.86 ± 0.03^{a}	17.12 ± 2.34^{c}	5.23 ± 0.72^{c}	42.34 ± 2.01^{e}
36 h	29.95 ± 4.61^{b}	18.00 ± 2.63^{c}	5.39 ± 0.63^{c}	41.46 ± 1.23^{e}
48 h	31.23 ± 2.41^{a}	18.13 ± 1.81^{c}	5.66 ± 0.56^{c}	37.60 ± 2.11^{e}
Citric acid				
12 h	18.83 ± 1.65^{c}	24.91 ± 2.04^{b}	4.69 ± 0.38^{c}	48.30 ± 1.89^{d}
24 h	16.91 ± 3.97^{c}	24.70 ± 1.60^{b}	4.18 ± 0.22^{d}	53.91 ± 1.05^{cd}
36 h	16.00 ± 3.48^{c}	24.17 ± 1.50^{b}	3.86 ± 0.24^{d}	57.44 ± 3.21^{c}
48 h	17.95 ± 0.49^{c}	25.04 ± 0.62^{a}	4.80 ± 0.11^{c}	47.08 ± 2.78^{d}
Hydrochloric acid				
12 h	7.88 ± 0.51^{d}	6.25 ± 1.13^{d}	0.49 ± 0.09^{e}	84.01 ± 5.32^{b}
24 h	12.64 ± 3.28^{d}	5.64 ± 0.40^{e}	0.79 ± 0.05^{f}	91.29 ± 4.23^{a}
36 h	11.44 ± 3.63^{e}	4.63 ± 0.23^{e}	0.79 ± 0.05^{f}	91.29 ± 1.36^{a}
48 h	13.82 ± 3.31^{d}	5.64 ± 0.28^{e}	0.78 ± 0.04^{f}	89.75 ± 2.15^{a}
Lactic acid				
12 h	30.48 ± 2.40^{a}	25.18 ± 1.01^{ab}	7.93 ± 0.32^{a}	12.61 ± 0.45^{g}
24 h	30.74 ± 0.03^{a}	24.51 ± 0.91^{b}	7.58 ± 0.28^{b}	16.43 ± 1.23^{f}
36 h	30.88 ± 0.45^{a}	28.40 ± 0.57^{ab}	7.72 ± 0.18^{a}	14.88 ± 1.12^{f}
48 h	30.42 ± 2.23^{a}	30.00 ± 1.39^{a}	7.77 ± 0.45^{a}	14.33 ± 1.04^{f}

n=10.

[1] Values are mean ± standard deviation

[a,b,c,d,e,f]: Means within the same column without the same superscript are significantly different ($p<0.05$).

collagen declines. The pH values of chicken feet with different acids and soaking times are shown in table 4. The pH values of chicken feet soaked with 5% acetic acid for different soaking times ranged from 3.44 to 3.62, and was significantly higher ($p<0.05$) than that of treatments with citric acid (2.43-2.63) and lactic acid (2.54-2.72). The pH of chicken feet soaked with hydrochloric acid wasn't determined in this experiment due to a very low pH value in this treatment. The relationship of pH and swelling percentage had been reported by several workers. Rose (1968) stated that bovine skin swelled with 2% lactic acid at pH 2.5-3.7 for 24-48 h. Chow (1981) also indicated that the largest swelling percentage of pig skin was at pH 2.5 (5% lactic acid) and 12.5, 17°C for 48 h. The results of the present study were agreed with the results of the previous researchers. No differences were found among pH value and swelling percentage of chicken feet after soaking 36 h with any acid treatments (tables 3 and 4).

Yield, collagen content, collagen/100 g chicken feet and collagen loss

The data of yield, collagen content, collagen/ 100 g chicken feet and collagen loss of chicken feet from various 5% acids and soaking times are shown in table 5. The yield

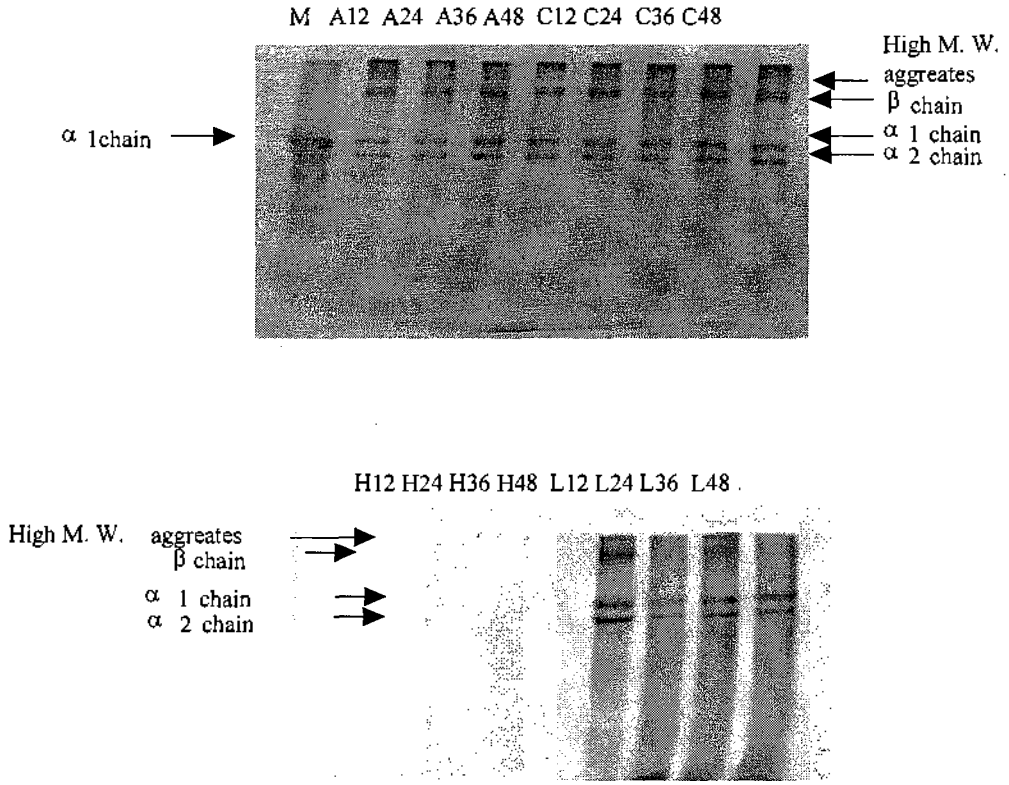

Figure 1. SDS-gel electrophoretogram of collagen from chicken feet treated with 5% different acids (A-acetic acid, C-citric acid, L-lactic acid and H-hydrochloric acid) for 12, 24, 36 and 48 h, individually. M- collagen type II (Sigma, Co.)

of crude collagen from acetic acid and lactic acid treatments were significantly higher ($p<0.05$) than that of the other two treatments. From table 5, the lowest crude collagen yield was found from hydrochloric acid at various soaking times. Wang (1994) stated that collagen can be digested or broken down yielding amino acids and peptides by concentrated hydrochloric acid, and the digestive degree wasn't controlled well. Therefore, in this study a large collagen loss (84.01-91.29%) from chicken feet with 5% hydrochloric acid treatment was obtained. This resulted in the smallest collagen yield. Based on the results of table 5, the best extraction condition for collagen was with the lactic acid treatment and the biggest extract amount of collagen from chicken feet was the ground chicken feet soaked with 5% lactic acid for 36 h.

Color of chicken feet collagen

The color of chicken feet collagen was expressed as Hunter L-lightness, Hunter a-redness and Hunter b-yellowness in this study. The data of table 6 indicated that Hunter L value of the samples from all treatments increased but the Hunter a values decreased with increased soaking time. Simultaneously, Hunter b value of the samples remained stable during soaking. These results indicated that the samples with a longer soaking time increased in lightness but was slightly red on the surface. Also a brighter yellow color was observed with a longer soaking time among acid treatments.

SDS-PAGE for collagen type

Bailey and Light (1989) stated that all collagen types consisted of three polypeptide chains (α-chains) in close association. They also evidenced that when acid soluble collagen from rat tail tendon was subjected to electrophoresis in order to separate the α-chains dimers and β chains, are clearly observed, and had two α-chains (α1-chain and α2-chain). In this study, collagen type II was used as a marker because it only contained three α1 chains. According to the components (bands) on the electrophoretogram (figure 1), it seem that collagen from chicken feet consisted of two types collagen, one is type I and another is type II. O'driscoll et al. (1985) also pointed out that the mixture of collagen type I and type II can be accurately identified by SDS gels. Two α chains dimers, one β chains and high molecular weight aggregates were clearly observed in all samples except for the samples treated with 5% hydrochloric acid for various soaking times from electrophoretogram (figure 1). This result indicated that collagen of chicken feet was markedly digested by 5% hydrochloric acid during swelling period. These results also suggested that a lower collagen yield content and a higher

Table 6. Color (Hunter L, a and b value)[1] of collagen from chicken feet treated with 5% different acids and soaking times

Time(h)/acid	Acetic acid	Citric acid	Hydrochloric acid	Lactic acid
L value				
12	70.18 ± 5.82^{bz}	69.48 ± 2.69^{bz}	74.74 ± 0.60^{az}	69.20 ± 0.93^{cy}
24	70.01 ± 2.65^{az}	68.58 ± 0.92^{az}	63.21 ± 0.78^{bx}	68.22 ± 1.06^{az}
36	70.43 ± 3.78^{az}	69.89 ± 2.84^{az}	67.73 ± 1.28^{ay}	64.06 ± 1.78^{bz}
48	72.88 ± 3.98^{az}	69.83 ± 3.70^{az}	71.40 ± 3.68^{az}	66.85 ± 0.52^{bz}
a value				
12	3.36 ± 1.55^{bz}	4.31 ± 1.26^{bz}	3.18 ± 1.57^{by}	2.27 ± 0.51^{az}
24	2.16 ± 0.57^{cy}	4.34 ± 0.75^{bz}	5.57 ± 0.86^{az}	3.06 ± 0.88^{cy}
36	2.73 ± 0.58^{abx}	1.08 ± 0.30^{by}	2.96 ± 1.62^{aby}	3.96 ± 1.42^{ay}
48	2.66 ± 1.85^{bx}	1.74 ± 0.80^{by}	2.23 ± 1.65^{by}	4.47 ± 0.67^{ay}
b value				
12	17.14 ± 0.60^{bz}	18.87 ± 0.68^{az}	17.72 ± 0.59^{byz}	18.98 ± 0.40^{az}
24	17.56 ± 1.74^{cz}	18.71 ± 0.39^{bz}	17.25 ± 1.46^{cy}	19.27 ± 0.17^{az}
36	17.41 ± 0.86^{bz}	17.55 ± 0.31^{by}	18.29 ± 0.61^{az}	19.04 ± 0.27^{az}
48	16.25 ± 0.27^{by}	15.76 ± 2.72^{bx}	18.66 ± 0.55^{az}	18.88 ± 0.19^{ay}

n=10.

[1] Values are mean ± standard deviation.

[a,b,c]: Means within the same column and item without the same superscript are significantly different ($p<0.05$).

[x,y,z]: Means within the same row and item without the same superscript are significantly different ($p<0.05$).

collagen loss was found in HCl treatment in this study.

CONCLUSION

In summary, the collagen amount of chicken feet was 9.07%. The amino acid composition of chicken feet was found that 30% of the total amino acid residue consist of glycine, approximately 11.7% of proline and 10-12.7% of alanine and glutamic acid. The swelling percentage of chicken feet soaked with citric acid (247%) and lactic acid (246.5%) was significantly higher ($p<0.05$) than that of the samples soaked with acetic acid (227.8%) and hydrochloric acid (129.91%). However, the optimum condition of extracting collagen from chicken feet was soaked with 5% lactic acid for 36 h due to its higher swelling percentage, yield of collagen and less collagen loss. A trace amount of type II collagen could be seen on electrophoretogram of collagen from chicken feet by various acid treatments except for hydrochloric acid.

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REFERENCES

A.O.A.C. 1995. Official Methods of Analysis (16th Ed.). Association of Official Analytical Chemists. Arlington, VA., USA.

Asghar, A. and R. L. Henrickson. 1982. Chemical, biochemical, functional and nutritional characteristics of collagen in food systems. Advances in food Research 28:232-312.

Bailey, A. J. and N. D. Light. 1989. Connective tissue in meat and meat products. Elsevier Science Publishers Ltd. London and New York pp. 75-119.

Balian, G and J. H. Bowes. 1977. The structure and properties of collagen. From The Science and Technology of Gelatin. Edited by Ward, A. G. and A. Courts, Academic press Inc. New York, USA. pp. 1-27.

Burgos, A., J. I. Floyd and E. L. Stephenson. 1974. The amino acid content and avialability of different samples of poultry by-product meal and feather meal. Poult. Sci. 53:198-203.

Chow Chi-Fa. 1981. Studies on using pork skin collagen to manufacture edible film. Master thesis, National Taiwan University, Taipei, Taiwan, ROC.

Eastoe, J. E. and A. A. Leach. 1977. Chemical constitution of gelatin From The Science and Technology of Gelatin. Edited by Ward, A. G. and A. Courts, Academic press Inc. New York, USA. pp. 73-83.

Ichie, K., Y. Taguchi, Y. Takahata, S. Toki, F. Morimatus, T. Yamanaka, T. Kumagai, Y. Wataya, K. Kimura, Y. Tanaka, K. Ikeda, A. Umetsu, R. Shibata, J. Kurisaki and R. Yamada. 1999. Low allergenic gelatin prepared from chicken cartilage by acid processing. 45th ICoMST, Japan, Proceeding 2:714-715.

Li, Shu-Tung. 1993 Collagen biotechnology and its medical application. Biomed Eng Appl Baia Comm. 5:646-657.

O'Driscoll, S. W., R. B. Salter and F. W. Keeley. 1985. A method for quantitative analysis of ratio of types I and II collagen in small samples of articular cartilage. Analytical Biochemistry 145:277-285.

Okerman, W. H. 1984. Quality control of post-mortem muscle tissue. The Ohio State University, Ohio, USA p. 51.

Pachence, J. M. 1992. Process for extracting type I collagen from an avian source, and applications therefor. US. Patent. 5138030.

Phadke, K., R. Fouts and J. E. Parish. 1984 Collagen-induced and adjuvant-induced arthritis in rats. Arthritis and Rheumatism 27(7):797-806.

Reddy, G. K. and C. S. Enwemeka. 1996. A simplified method for the analysis of hydroxyproline in biological tissue. Clin. Biochem. 29:225-229.

Rose, H. J. 1968 Method of preparing an edible tubular collagen casing. U.S. Patent 3413130.

SAS Institute Inc. 1989. SAS Users Guide: Statistics. Version 7 Ed. Cary, NC., USA.

Taguchi, Y., T. Matsumoto, K. Fujita, F. Morimatus and T. Shigchisa. 1998. Oral administration of type II collagen from chicken cartilage suppresses adjuvant arthritis in rats. 44th CoMST, Spain, Proceeding 2:1030-1031.

Tanaka, C. Y. and M. Shimokomaki. 1996. Collagen type in mechanically deboned chicken meat. J. Food Biochem. 20:215-225.

Todbunter, Rory J., A. M. Wootton, and R. R. Minor. 1994. Structure of equine type I and type II collagens. Am J Vet Res 55(3):425-431.

Trentham, D. E., R. A. Dynesius-Trentham, E. J. Orav, D. Cormbitchi, C. Lorenzo, K. L. Sewell, D. A. Hafler and H. L. Weiner. 1993. Effect of oral administration of type II collagen on rheumatoid arthritis. Science 261:1727-1730.

Wang, D. 1994. Studies on manufacturing a functional ingredient from porcine skin collagen by enzyme hydrolysis. Master thesis. Tunghai University, Taichung, Taiwan, ROC.

Yoshino, S. 1996. Oral administration of type II collagen supresses non-specifically induced chronic arthritis in rats. Biomed and Pharmacother 50:24-28.

Zhang, Z. J., S. Y. Lee and H. L. Weiner. 1990. Suppression of adjuvant arthritis in lewis rats by oral administration of type II collagen. The Journal of Immunology 145:2489-2493.