PE4.49 The effect of different acids on the extraction of gelatin from chicken feet 175.00

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***Abstract*—The physicochemical characteristic of gelatin obtained by different pretreatment of chicken feet with 0.1M and 0.5M acetic acid, citric acid, hydrochloric acid, lactic acid, phosphoric acid solutions have been studied. The resulting gelatin from chicken feet treated with different acid solutions was evaluated for its swelling percentage and extracting yield of chicken feet and gel strength, collagen content, color, of gelatins. Among five acid extractions, the lactic acid had both the highest swelling percentage (185%, 174%) and extracting yield (7.20%, 8.62%) in 0.1 and 0.5M acid solution, respectively, while the hydrochloric acid was the lowest medium for chicken feet gelatin extraction. Acetic or lactic acid treatment resulted higher collagen content among treatments. According to the result of yield, collagen and gel strength, the best condition of extracting gelatin from chicken feet was soaked in lactic acid and phosphoric acid in 0.1M acid solution and lactic acid in 0.5M acid solution. However, a higher lightness color of gelatin from all treatments was observed with low acid solution concentration.**

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***Index Terms*—chicken feet, gelatin, extraction, collagen, gel strength**

I. INTRODUCTION

CHICKEN contain large amount of collagen, which can be easily converted into gelatin upon heating. Some are used either processed or unprocessed, as feed for livestock (Cha, Cho, Chung, Chang, & Yi, 1995), while very little portion is used for human consumption. Few studies have been 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, Lee, Lee, & Kim, 2000). Gelatin is a fibrous protein that is extracted from collagen and is an important functional biopolymer that has a very broad application in many industrial fields, such as food, materials, pharmacy and photography. It is especially useful in the food and pharmaceutical industries for its unique chemical and physical characteristics (Rahman, Al-saidi, & Guizani, 2008). Chicken feet, cattle hides and pork skins are the traditional materials used in gelatin production (Cho, Gu & Kim, 2005; Liu, Lin, & Chen, 2001; Sobral & Habitante, 2001). Acid extraction with hydrolysis was a common method for collagen extraction in recent time. Specially, acetic acid has frequently been used as a solvent for collagen extraction and a number of collagen extractions. (Nagai, Izumi, & Ishii, 2004). The purpose of this study was to determine the effect of pretreatment on gelatin extraction from chicken feet and the physicochemical characteristics of the gelatin obtained to provide a possible approach for utilization of chicken feet.

II. MATERIALS AND METHODS

1. Preparation of chicken feet

Frozen chicken feet were soaked in water at room temperature (25±1ºC) for 3 h to remove blood. After being washed with distilled water, the chicken feet were treated with 5 vol. (v/w) of 0.1M and 0.5M various acid solutions (acetic acid, citric acid, hydrochloric acid, lactic acid, phosphoric acid) and soaked for 48 h at 20±1ºC. After pretreatment, the chicken feet were rinsed with distilled water and was supplied every 3 h to remove impurities and acidity for 1 day.

2. Extraction of gelatin from chicken feet

The pretreated chicken feet were extracted at 80ºC for 2hr. The extracted solution was filtered through double layer gauze and Whatman filter paper (No. 4). The extraction solutions were diluted with distilled water to a protein concentration of 50 mg/mL for analysis of gel strength, collagen content, and color.

3. Analysis of gelatin from chicken feet

3.1. pH value and swelling percentage measurement

The pH of sample was determined using a pH meter (Model 340, Mettler-Toledo GmbH, Schwerzenbach, Switzerland). Swelling percentage was expressed as: (the weight of the residue at the soaking time using different acids/the weight of chicken ground feet before soaking) × 100.

3.2. Gelatin yield

Protein concentration was determined by the Biuret method, and the extraction yield was calculated by the following equation: Yield (%) = 100 × (protein concentration in g/mL) × (volumn of extracts in mL) / weigh of raw material.

3.3. Color measurement

The color of the gelatin was determined by measuring the lightness, redness and yellowness values (L^*, a^*, and b^*) using a colorimeter (Chroma meter DP-400, Minolta, Japan; illuminate C, calibrated with white standard plate $Y = 93.5$, $X = 0.3134$, $Y = 0.3197$). Color values (CIE L^*, a^*, and b^*) were measured on the surface of samples.

3.4. Gel strength

The gel strength was determined using a Texture Analyzer (TA-XT2*i*, Stable Micro System Ltd., Surrey, U.K.). A gelatin mixture was poured into a plastic container 40×40×25 mm), which was placed in a chill bath (4 ºC), for 15 h to gel. The gel strength for each gelatin was measured by a spherical probe (φ 0.25 mm diameter). Test speed was set at 2 mm/sec, and lad cell at 10 kg. The samples (height 25mm) were compressed to 60% strain of the original vertical height.

3.5. Collagen determination

The collagen content was determined based on the Kolar (1990) and basically 4 g of sample was hydrolyzed with 7N H2SO4 at 105 °C for 16 h. Hydroxyproline concentration was determined by reacting with p-dimethylaminobenzaldehyde.

Table 1. The swelling percentage and extracting yield of chicken feet using different acids

Treatment [a]		Swelling percentage (%)	Extracting yield (%)	pH value of solution [b]
0.1M	A	141.95±7.71^{C}	4.81±0.38^{D}	3.82±0.01^{A}
	C	171.07±3.07^{B}	5.66±0.37^{C}	2.75±0.02^{C}
	H	135.85±2.73^{C}	6.57±0.14^{B}	1.61±0.04^{E}
	L	185.42±8.10^{A}	7.20±0.15^{A}	3.01±0.02^{B}
	P	179.28±14.72^{A}	7.29±0.17^{A}	1.85±0.03^{D}
0.5M	A	163.30±1.61^{B}	6.19±0.09^{C}	3.33±0.03^{A}
	C	177.80±2.12^{A}	7.61±0.45^{B}	2.12±0.04^{C}
	H	105.92±2.35^{D}	5.55±0.04^{D}	0.89±0.02^{E}
	L	174.31±14.23^{A}	8.62±0.30^{A}	2.53±0.03^{B}
	P	134.25±4.77^{C}	7.64±0.31^{B}	1.42±0.01^{D}

All values are mean ± SD of the three replicates.
[A-E] Means sharing different letters in the same column are significantly different ($p < 0.05$).
[a] Different acid solution; A:acetic acid, C:citric acid, H:hydrochloric acid, L:lactic acid, P:phorsphoric acid.
[b] pH values were determined at the final acid treatment procedure.

3.6. Statistical analysis

All the analysis was done at least three times under each experimental condition and mean values were reported. An analysis of variance were performed on all the variables measured using the General Linear Model (GLM) procedure of the SAS statistical package (SAS Institute, Inc., 1999). The Duncan's multiple range test ($p < 0.05$) was used to determine differences between treatment means.

III. RESULTS AND DISCUSSION

The extracting yield and swelling percentage of gelatin extracted from chicken feet by different acid solution are shown in Table 1. As soaking time increased, the swelling percentage significantly increased ($p<0.05$). Citric acid and lactic acid were effective solvents for swelling capacity. Lactic acid and phosphoric acid showed the significant ($p<0.05$) higher swelling percentage (185% and 179%) in 0.1M acid solution and citric acid and lactic acid showed the significant ($p<0.05$) higher swelling percentage (177% and 174%) in 0.5M acid solution. The chicken feet treated lactic acid (7.20 %) and phosphoric acid (7.29%) in 0.1M acid solution had the significantly higher extracting yield than other treatments and 0.5M lactic acid treatment also had the highest extracting yield among treatment with 0.5M acid solution. The extracting yields treated lactic acid and phosphoric acid were effective solvent for extracting yield from chicken feet. The pH values at the final acid solution treatment

(Table 1) might be explanation of why swelling percentage of chicken feet were obtained in citric acid and lactic acid treatment, where pH 2.12 ~ pH 3.01 were found in them.

Table 2. The gel strength and collagen contents of gelatin from chicken feet using different acids

Treatment [a]		Gel strength (g·cm)	Collagen content (mg/g)
0.1M	A	252.78±4.41^{B}	16.36±0.79^{C}
	C	235.77±10.00^{C}	22.98±1.67^{AB}
	H	223.46±5.16^{D}	15.07±1.14^{C}
	L	293.51±12.41^{A}	24.54±1.14^{A}
	P	202.94±11.17^{E}	22.33±.186^{B}
0.5M	A	247.50±8.32^{B}	18.57±0.67^{B}
	C	239.18±10.93^{B}	18.38±0.97^{B}
	H	207.49±9.28^{C}	14.43±1.32^{C}
	L	281.92±8.74^{A}	21.32±1.36^{A}
	P	194.05±7.74^{D}	17.37±0.63^{B}

All values are mean ± SD of the three replicates.
[A-E] Means sharing different letters in the same column are significantly different ($p < 0.05$).
[a] Different acid solution; A:acetic acid, C:citric acid, H:hydrochloric acid, L:lactic acid, P:phorsphoric acid.

Extracting yield and swelling percentage were related to pH value of solutions. The lactic acid treatment showed the higher swelling percentage seemed to contribute the extracting yield, while the hydrochloric acid was the lowest medium for chicken feet gelatin extraction.

The gel strength of gelatins obtained from the chicken feet treated different acid solution, after maturation overnight at 4 ºC is shown in Table 2. When acid concentration was increased, their gel strength of gelatin was decreased in all treatments. The results showed that lactic acid and acetic acid treatment obtained higher gel strength. And the gelatin treated phosphoric acid solution had the lowest gel strength (p<0.05). The result of collagen content of gelatin from chicken feet treated with different acid solutions is shown in Table 2. Hydrochloric acid was the least effective solvent for collagen extraction due to its lowest pH value, while acetic or lactic acid treatment resulted higher collagen content.

The color of gelatin from chicken feet is showed Table 3. Gelatin produced from 0.1M acid pretreatment generally had higher L^*-value than those produced from other acid solution. As increased acid solution concentration increased lightness. a^*-value of gelatin treated lactic acid was the highest value among 0.1M solution treatments and the gelatin in acetic acid was the highest a^*-value among 0.5M solution treatments.

Table 3. The color properties of gelatin from chicken feet using different acids

Treatment [a]		Color of gelatin		
		L^*	a^*	b^*
0.1M	A	43.71±1.09^{B}	-0.11±0.07^{D}	1.51±0.30^{B}
	C	46.68±2.25^{A}	0.31±0.02^{B}	2.47±0.17^{A}
	H	41.46±1.37^{C}	0.28±0.06^{B}	1.41±0.18^{B}
	L	47.53±1.70^{A}	0.40±0.04^{A}	1.53±0.10^{B}
	P	42.47±2.03^{B}	0.11±0.10^{C}	2.70±0.28^{A}
0.5M	A	43.81±0.63^{A}	0.31±0.04^{A}	1.33±0.16^{C}
	C	40.89±1.07^{B}	0.17±0.09^{BC}	2.40±0.18^{A}
	H	38.98±1.39^{C}	0.10±0.08^{C}	2.09±0.17^{AB}
	L	40.12±0.90^{B}	0.23±0.05^{AB}	1.80±0.16^{B}
	P	41.04±1.79^{B}	0.15±0.07^{BC}	2.19±0.46^{A}

All values are mean ± SD of the three replicates.
[A-D] Means sharing different letters in the same column are significantly different ($p < 0.05$).
[a] Different acid solution; A:acetic acid, C:citric acid, H:hydrochloric acid, L:lactic acid, P:phorsphoric acid.

IV. Conclusion

Based on the studies above, gelatin is possible to obtain from chicken feet using different acid solution. The lactic acid and citric acid treatment showed the highest yield content and swelling percentage, while the hydrochloric acid was the lowest medium for chicken feet gelatin extraction. The swelling percentage and extracting yield related with pH value of acid solution. The best extraction condition for collagen was with the lactic acid treatment. The finding of this work will contribute to the utilization of industrial wastes.