THE SOAKING EFFECT ON DIFFERENT HYDROCLORIDE ACID LEVEL AND SOAKING TIME ON PH, SWELLING PERCENTAGE AND COLLAGEN YIELD OF CHICKEN SHANK BONE

D.A.P. Puspitasari, V.P. Bintoro, B.E. Setiani
Faculty of Animal and Agricultural Sciences, Diponegoro University
Tembalang Campus, Semarang 50275 - Indonesia
Corresponding E-mail: dyahayu.pediatrika@yahoo.com

Received March 18, 2013; Accepted May 12, 2013

ABSTRAK

Kolagen merupakan bahan utama dalam pembuatan produk gelatin. Tujuan dari penelitian ini adalah untuk mengetahui nilai pH, persentase *sweeling* dan rendemen kolagen tulang cakar ayam yang direndam dengan konsentrasi HCl dan lama perendaman yang berbeda. Nilai pH dan *persentase sweeling* tulang cakar ayam merupakan indikator yang menentukan jumlah rendemen kolagen. Rancangan penelitian yang digunakan adalah rancangan acak lengkap (RAL) faktorial, dengan faktor A = konsentrasi HCl (2%, 3,5% dan 5%) dan faktor B = lama perendaman (24, 36 dan 48 jam) tanpa dilakukan pengulangan (profil). Berdasarkan hasil penelitian seiring dengan meningkatnya konsentrasi HCl dan lama perendaman akan menurunkan nilai profil pH (0,17 – 0,85) dan profil *persentase sweeling* (80,25 – 97,49%) sehingga menghasilkan profil rendemen kolagen yang rendah (1,80 – 10,02%). Rendahnya kolagen tulang cakar ayam yang dihasilkan diduga diakibatkan oleh asam pengekstraksi yang terlalu kuat (HCl). Hasil rendemen kolagen tulang cakar ayam tertinggi diperoleh dari perendaman 2% HCl selama 24 jam. Berdasarkan hasil penelitian, kolagen tulang cakar ayam dapat direkomendasikan menjadi salah satu bahan baku gelatin.

Kata Kunci : asam klorida, kolagen, lama perendaman, tulang cakar ayam

ABSTRACT

Collagen is the main ingredient in the gelatin manufacture. The purpose of the study was to determine the pH value, the swelling percentage and collagen yield of chicken shank bones which were soaked by different HCl concentration and soaking time. The pH value and swelling percentage of chicken shank bones are indicator that determines amount of collagen yield. The research design used was factorial completely randomized design (CRD). Factor A was a concentration of HCl (2%, 3.5% and 5%) and factor B was a soaking time (24, 36 and 48 hours) without replication (profile). The result shows that the the increase of HCl concentration and soaking time affected on the decreasing of pH value profile (0.17-0.85), swelling percentage profile (80.25-97.49%) and also collagen yield (1.80 -10.02%). The lowest collagen yield of chicken shank bones was suspected caused by the used of Hcl. The highest result of the study was using 2% of HCl concentration for 24 hours. The best result was found in the use of 2% HCl and was soaked for 24 hours. Based on those result, the collagen of chicken shank bones was recommended as a main ingredient for gelatin manufacture.

Keywords : chicken shank bones, hydrochloric acid, collagen, soaking time

INTRODUCTION

Chicken shank as a byproduct of poultry slaughter house unutilized optimally yet (Miwada and Simpen, 2007). However, chicken shank contains a lot of collagen about 5.64-31.39% (Liu *et al*., 2001) or 28.73-36.83% of total proteins (Prayitno, 2007). Collagen is the main ingredient of gelatin manufacture. Collagen has triple helix structure and will change into single helix when treated by acid or alkaline. Collagen structure will swell and change into single helix when the pH is lower than 4 or higher than 10. This condition causes collagen chain become longer, dissolve into the water and it would be changed into gelatin solution when heated. Gelatin solution

would be changed into gelatin when the hydro gel randomly returns to triple helix chain in low temperature (cooled) (Li, 1993; Prayitno, 2007;Yuniarifin *et al*., 2006). Gelatin can be used for photographic industry, pharmaceutical and food industry as emulsifier, foaming agent, stabilizer, water binding, edible film and microencapsulation depends on the forming gel ability (Hidaka and Liu, 2003; Miwada and Simpen, 2007; Guillen *et al*., 2011).

In previous study, chicken shank collagen had been observed in whole of chicken shank, but collagen from chicken shank bones has not been identified clearly until now. Therefore, the objectives of this study were to determine the pH value, swell percentage and collagen yield of chicken shank bones soaked by different HCl concentration and soaking time.

MATERIALS AND METHODS

Materials

The materials used for the study were 450 g of chicken shank bones prepared from 5 kg of chicken shank bones which were then divided into 50 g for 9 treatments.

Treatments

The treatments were consisted of a_1b_1 = soaked with 2% of HCL for 24 hours, a_2b_1 = soaked with 3.5% for 24 hours, a_3b_1 =soaked with 5% for 24 hours, a_1b_2 = soaked with 2% for 36 hours, a_2b_2 = soaking by HCl 3.5% with 36 hours, a_3b_1 = soaking by HCl 5% with 36 hours, a_1b_3 = soaking by HCl 2% with 48 hours, a_2b_3 = soaking by HCl 3.5% with 48 hours, a_3b_3 = soaking by HCl 5% with 48 hours. Approximately 3 cm of chicken shank bones were soaked in 2%, 3.5% and 5% of HCl for 24, 36 and 48 hours according to the treatment. After being soaked, the treated chicken shank bones in suspended solution from each treatment were homogenized for 5 minutes and the bones residue was discarded using paper filtration. The suspended solutions were then being neutralized - the pH value reached 7 by using 0.1 N NaOH. The neutral solution was then homogenized by using centrifuge for 15 minutes in the high speed. The supernatant was discarded and the precipitate was lyophilized by dryer to obtain a dry collagen (Liu *et al*., 2001). The following step was measuring collagen yield and swelling percentage of dry collagen by following formula:

Sweelling Percentage = [weight of total solid after soaking/weight of bone before soaking] x 100%

Collagen yield = [weight of dry collagen/ weight of bone before soaking] x 100%

Statistical Analysis

The statistical design applied in the study was completely randomized design (CRD), factorial pattern with 2 factors without replication (profile), in which A factor was extraction acid concentration (HCl 2%, 3.5% and 5%) and B factor was soaking time (24 hours, 36 hours and 48 hours).

RESULTS AND DISCUSSION

The chemical compositions of chicken shank bones

The chemical compositions of chicken shank bones which were soaked by 2-5% HCl for 24-48 hours shown in Table 1. The table shows that chicken shank bones contain 61.47% of moisture, 1.16% of protein, 7.18% of fat and 14.51% of ash. The result indicated that chicken shank bones have lower protein content than bovine and red snapper fish bones. The data showed that chicken shank bones have 1.16% proteins, while bovine and fish bones have 15.46% and 23.51% (Hajrawati, 2006 and Kusumawati *et al*., 2008). According to Liu *et al*. (2001), chicken shank has lesser proteins content (17.42 %) than calf skin (30 - 35%) and pig skin (31%). The lesser proteins content showed that chicken shank bones have a lot of ash. The ratio of proteins and ash was 1:12. Field *et al*. (1974) stated that ash of chicken bones was 5.26% and contains 39.08% of calsium. According to Liu *et al*. (2001), the high level of ash content indicated that the bones contain a lot of residue. Bovine bones consist of 7.68% moisture, 15.46% protein, 39.86% fat and

Table 1. Chemical Compositions of Chicken Shank Bones

Chemical Composition	Content (%)
Moisture	61.47
Protein	1.16
Fat	7.18
Ash	14.51

34.81% ash (Hajrawati, 2006). Red snapper fish (*Lutjanus* sp.) bones consist of 9.23% moisture, 23.51% protein, 5.02% fat and 49.51% ash (Kusumawati *et al*., 2008).

pH Value Profile

The pH of chicken shank bones (CSB) which was soaked by 2-5% of HCl concentration for 24-48 hours is shown in Table 2. Table 2 shows that the highest HCl concentration and the longest soaking time result in lowering the pH value. The pH of CSB soaked by 2-5% of HCl for 24-48 hours was 0.17 - 0.85. The highest pH value was obtained by chicken shank bones soaked by 2% of HCl for 36 hours (0.85 pH value).

The lowering pH of chicken shank bones along with the increasing of HCl concentration and soaking time was suspected caused by much of H^+ contained in CSB. According to Ward and Courts (1977) and Kolodziejska *et al.* (2007), increasing acid concentration also resulted in the increase of H^+ in the acid solution. Increasing the number of H^+ can accelerate the rate of collagen hydrolysis. Liu *et al*. (2001) stated that CSB soaked with 5% of HCl for 12-24 hours has lower pH value than those soaked with 5% of citric acid concentration (2.43-2.63), lactic acid concentration (2.54-2.72) and asetic acid concentration (3.44-3.62). Hydrochloric acid classified as a strong acid which has valency –1. Therefore, soaking of bones in hydrochloric acid would result in lower pH than using other acid. Jongjareonrak (2006) reported that three factors affected the collagen functional properties: aging and the living period of samples, the processing steps, the pH and NaCl concentrations during the collagen extraction step.

The Swelling Percentage Profile

The swelling percentage of chicken shank bones (CSB) which were soaked by 2- 5% of HCl concentration for 24-48 hours is shown in Table 2. The Table 2 shows that the highest HCl concentration and the longest soaking time result in lowering swelling persentage of CSB. The CSB which were soaked by 2-5% of HCl concentration for 24-48 hours had 80.25-97.49% of swelling percentage. The highest swelling percentage of CSB was obtained by those soaked in 5% of HCl concentration for 48 hours (97.49% swelling percentage). The swelling percentage was affected by pH value. The highest of HCl concentration and the longest of soaking time would decrease pH value, and increase the yield of collagen and swelling percentage. According to Li (1993), collagen had swelled when the range of pH value was bellow 4-above 10. On this pH range, the collagen chain has weak binding ability.

Collagen Yield Profile

The collagen yield of chicken shank bones (CSB) which were soaked by 2-5% of HCl concentration for 24-48 hours is shown in Table 2. Table 2 shows that the highest HCl concentration and the longest soaking time result in lowering the collagen yield. The collagen yield of CSB soaked

Table 2. The Soaking Effect Caused by Different HCl Concentration and Soaking Time on pH, Collagen Yield and Swelling Percentage Profile of Chicken Shank Bones

Parameters	HCl Content	Soaking Time (hours)		
		24	36	48
pH	2.0	0.77	0.85	0.72
	3.5	0.45	0.59	0.36
	5.0	0.31	0.17	0.18
Yield of Collagen (%)	2.0	2.87	7.55	10.02
	3.5	7.29	1.80	3.97
	5.0	3.00	2.56	4.92
Swelling Percentage (%)	2.0	94.90	91.94	91.02
	3.5	97.43	83.37	91.53
	5.0	94.92	80.25	97.49

by 2-5% of HCl concentration for 24-48 hours was 1.80-10.02. The highest collagen yield was obtained by CSB soaked in 2% of HCl concentration for 48 hours (10.02% collagen yield).

Table 2 shows that the highest HCl concentration and the longest soaking time applied in CSB would produce–unstable collagen extracted. Based on the data, pH value influenced collagen yield. When HCl concentration and soaking time increases, the pH value decreases as well as the yield, except soaked by 5% of HCl concentration for 36 hours. The phenomenon showed that HCl has no spesific properties, irreguler and uncontrol. Study done by Liu *et al*. (2001) showed that HCl had lower ability to extract whole of chicken shank collagen than acetic acid, lactic acid and citric acid did. HCl has the ability to brake down peptide and amino acid and could not be controled. However, a study by Prayitno (2007) revealed that HCl had similar capabilities with lactic acid to extract whole of chicken shank collagen.

The CSB soaked by 2-5% of HCl concentration for 24-48 hours suspected has produced type I collagen. According to Gelse *et al*. (2003), Muyonga *et al*. (2004), Prayitno (2007) and Guillen *et al*. (2011), types I and V collagen fibrils contribute to the structural backbone of bone and types II and XI collagens predominantly contribute to the fibrillar matrix of particular cartilage. Kolodziejska *et al*. (2008) reported that temperature was correlated with the collagen extraction yield. The solubilization of collagen increased along with the increasing of temperature and completely solubilized at 45°C. Potaros *et al*. (2009) and Guillen *et al*. (2011) stated that collagen is composed of three similarly sized of triple helix polypeptide chains. Collagen has a repetitive primary sequence of which every third residue is glycine. The amino acid content was correlated in the same direction as the denaturation temperature. If the extractives were rich in amino acids, the denaturation temperature was high too. The amino acids have a tropocollagen triple-helix structure and can form a strong hydrogen bond with surrounding molecules leading to increase collagen heat stability and a higher denaturation temperature.

CONCLUSION

Chicken shank bones has lesser proteins content than bovine bone and red snipper fish bone. The chicken shank bones which were soaked in the highest HCl concentration and the longest soaking time had lowered pH value profile (0.17-0.85), swelling percentage profile (80.25-97.49%) and collagen yield profile (1.80-10.02%). Along with the increase of HCl concentration and soaking time resulted the collagen of chicken shank bones extracted process into non-specific and were not controlled, so that the production of collagen yield was low. The highest results of the study were treatments using 2% of HCl concentration for 24 hours time. Based on those result, the collagen of chicken shank bones was recommended as a raw material for gelatin manufacture.

REFERENCES

Gelse, K., E. Poschl and T. Aigner. 2003. Collagens – structure, function and biosynthesis. Adv. Drug Delivery Rev. 55:1531--1546

Guillen, M.C.G., B. Gimenez., M.E.L. Caballero and M.P. Montero. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources. Food Hydrocolloids. 25:1813-1827

Hajrawati. 2006. Sifat fisik dan Kimia Gelatin Tulang Sapi dengan Perendaman Asam Klorida pada Konsentrasi dan Lama Perendaman yang Berbeda. Thesis. Institut Pertanian Bogor, Bogor.

Hidaka, S. and S.Y. Liu. 2003. Effects of gelatins on calcium phosphate precipitation : a possible application for distinguishing bovine bone gelatin from porcine skin gelatin. J. Food Compos. Anal. 16:477-483

Jongjareonrak, A. 2006. Characterization and Functional Properties of Collagen and Gelatin from Bigeye Snapper (*Priacanthus macracanthus*) and Brownstripe Red Snapper (*Lutjanus vitta*) Skins. Ph.D. Thesis. Prince of Songkla University.

Kolodziejska. I., E. Skierka, M. Sadowska. W. Kolodziejska and C. Niecikowska. 2008. Effect of extracting time and temperature on yield of gelatin from different fish offal. Food Chem. 107: 700-706

Kusumawati, R., Tazwir and A. Wawasto. 2008. Pengaruh perendaman dalam asam klorida terhadap kualitas gelatin tulang kakap merah. Jurnal Pascapanen dan Bioteknologi dan Perikanan. 3(1):63-68

Miwada, I. N. S and N. Simpen. 2007.

Optimalisasi potensi ceker ayam (*shank*) hasil limbah rpa melalui metode ekstraksi termodifikasi untuk menghasilkan gelatin. Majalah Ilmiah Peternakan. 10(1): 5-8

Muyonga, J.H., C.G.B, Coleb and K.G. Duod. 2004. Characteristisation of acid soluble collagen from skins of young and adult nile perch (*Lates niloticus*). Food Chem. 85: 81 – 89.

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

Liu, D.C, Y.K. Lin, and M.T. Chen. 2001. Optimum condition of extracting collagen from chicken feet and its caracetristics. Asian-Aust. J. Anim. Sci. 14:1638-1644

Potaros, T., N. Raksakulthai, J. Runglerd-kreangkrai and W. Worawattanamateekul. 2009. Characteristic of collagen from nile tilapia *(Oreochromis niloticus)* skin isolated by two different methods. Kasetsart J. (Nat. Sci) 43: 584-593

Prayitno. 2007. Ekstraksi kolagen cakar ayam dengan berbagai jenis larutan asam dan lama perendaman. Anim. Prod. Jenderal Soedirman University. 9(2):99-104

Ward, A.G. and A. Courts. 1977. The Science and Technology of Gelatin. Academic Press, New York.

Yuniarifin, H., V.P. Bintoro, and A. Suwarastuti. 2006. Pengaruh Berbagai Konsentrasi Asam Fosfat Pada Proses Perendaman Tulang Sapi Terhadap Rendemen, Kadar Abu dan Viskositas Gelatin. J. Indonesian Trop. Anim. Agric. 31(1):55-61