International Journal of Multidisciplinary Education and Research
www.multidisciplinaryjournals.in
ISSN: 2455-4588
Received: 12-06-2024, Accepted: 06-07-2024, Published: 29-07-2024
Volume 9, Issue 3, 2024, Page No. 16-19

The effect of chloride Acid submersion with various concentrations on viscosity, pH, and gel strength of rabbit skins gelatine

Jajang Gumilar[1], Dania Mahardika[2], Lilis Suryaningsih[1]
[1] Department of Animal Product Technology, Faculty of Animal Husbandry, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang, Hegarmanah, Jatinangor, Sumedang, West Java, Indonesia
[2] Study Program of Animal Husbandry, Faculty of Animal Husbandry, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang, Hegarmanah, Jatinangor, Sumedang, West Java, Indonesia

Abstract
The demand for gelatin in Indonesia tends to increase along with the development of trends in consumption patterns. Currently, gelatin needs in Indonesia are still fulfilled by importing, leading to a polemic about its halal status. Another alternative ingredient in making gelatin is rabbits. Rabbit skin contains collagen which can be hydrolyzed into gelatin. This aim of the study is to assess the effect of employing various concentrations of hydrochloric acid on viscosity, pH, and gel strength. This research employed an experimental procedure with a Completely Randomized Design (CRD) with five treatments of hydrochloric acid concentration (1%, 2%, 3%, 4%, 5%) and four repetitions. Research data were examined using analysis of variance and then followed by Duncan's multiple range tests. The results showed that the effect of hydrochloric acid submersion with various concentrations had a significant effect ($P<0.05$) on the viscosity and gel strength of rabbit skin gelatin, but a non-significant effect ($P>0.05$) on the pH of rabbit skin gelatin. The best viscosity, pH, and gel strength of rabbit skin gelatin is 1% treatment which is 54,92 mps, 5,35, and 141,61 gram bloom.

Keywords: Gelatin, chloride acid, viscosity, pH, gel strength

Introduction

Gelatin is a collagen protein that was partially hydrolyzed from white connective tissue, bones, and skin of animals. Gelatin is a soluble protein that can act as a gelling or a non-gelling agent. Pure collagen is very sensitive to enzymatic and chemical reactions. The alkaline treatment causes collagen to expand and spread, which is often converted to gelatin. Besides alkaline solvents, collagen is also soluble in acidic solvents (Schriebert and Gareis, 2007) [25].

The general method for making gelatin is that animal bones or skin which are rich in collagen are soaked in base or acid, and then extracted using heat (Hastuti and Sumpe, 2007) [10]. The acid-soaking process in making gelatin causes the conversion of collagen into a form suitable for hydrolysis, namely by the interaction of H+ ions from the acid solution with collagen (Martianingsih and Amaja, 2010) [18].

Alkaline soaking solutions can only generate double chains, whereas acids can transform triple-helix collagen fibers into single chains (Junianto, *et al.* 2006) [13]. The use of weak acids does not increase H+ ions in the curing solution, so the hydrolysis rate does not increase the amount of conversion to gelatin (Sasmitaloka, *et al.* 2017) [24]. Soaking in an alkaline solution is considered less effective because the alkaline process takes a long time compared to the acid process to convert collagen into gelatin, this is because alkaline solutions can only decompose tropocollagen into double chains (Astawan and Aviana, 2003) [3].

HCl can break down collagen fibers at an increasing rate without impacting the quality of the gelatin produced and converts triple helix collagen fibers into single chains (Zhang, *et al.* 2010) [28]. The stronger the type of acid used, it will cause an increase in the amount of dissolved collagen and will even be lost during ossein washing, thus affecting the amount of yield produced (Mulyani, *et al.* 2012) [19]. If the acid concentration used is too high, the proteins contained in collagen cannot turn into gelatin (Jannah, 2007) [11].

The results of research conducted by Rapika, *et al.* (2016) [21] showed that 3% HCl concentration with a soaking time of 12 hours produced the highest values of viscosity and strength of cowhide gel, namely 1.79 cP and 185.00 g/Bloom, and the use of 3% HCl with the 4 hours soaking times produces the highest pH of cowhide, namely 4.12. Another research conducted by Gumilar and Pratama (2018) [7], showed that soaking in 3% HCl for 24 hours produced the highest pH value and gel strength compared to soaking in 5% and 7% HCl, namely 5.55 and 157.48 g/Bloom. In research conducted by Sasmitaloka, *et al.* (2017) [24], the viscosity and strength of dry cowhide gelatin gel soaked with 1% HCl produced the highest values of 8.25 ± 0.71 cP and 88.25 ± 0.96 bloom compared to soaking with CH_3COOH 1%, and NaOH 1%.

The use of livestock skins as raw material for gelatin has been widely studied. Several studies have used tanned cowhide (Nurhalimah, 2010; Sugihartono, 2014) [20], cowhide from slaughterhouses (Nurhalimah, 2010) [20], cowhide that has undergone the hair removal process (Youlanda, 2016) [27], dried cowhide (Sasmitaloka, *et al.* 2017) [24], sheepskin (Hasdar and Rahmawati, 2016) [9], goatskin (Said, *et al.* 2011) [22], and pigskin (Sompie, *et al.* 2012) [26]as raw materials for gelatin, but there has been no research on its use rabbit skin for making gelatin. This study tries to establish the quality of gelatin from rabbit skin using hydrochloric acid in a soaking process based on pH, viscosity, and gel strength.

Materials and methods

The skin used was local Indonesian rabbit skin. The tools used were analytical scales (Ohaus), drying oven (Julabo), water bath (Julabo), pH meter (Hanna), pycnometer, magnetic stirrer, Ostwald viscosity, TA-XT plus texture analyzer, and standard bloom jars.

Gelatin production

Gelatin production referred to the method developed by Said, *et al.* (2011) [22], starting from the degreasing stage by boiling the skin in boiled water (95º C) for 30 minutes, then cut it into 3 cm pieces. The demineralization stage is carried out by soaking the skin in 1%, 2%, 3%, 4%, and 5% HCl solution for 24 hours. The neutralization stage was carried out by washing the skin using running water until the pH was neutral. The extraction stage was carried out using a water bath at a temperature of 70ºC for 5 hours. The filtering stage was carried out by pouring the solution into a glass covered by Whatman filter paper no. 42. The drying stage was carried out using a drying oven at a temperature of 50ºC for 42 hours. After that, the gelatin was powdered by blending and then analyzed further to determine the quality of the gelatin produced.

Viscosity

The gelatin solution has a 6.67 percent (w/w) concentration was heated to 60°C and then tested using a calibrated capillary pipette. The efflux time is calculated in seconds and converted to milli poise based on the relationship determined during calibration. Each gelatin viscosity pipette is calibrated using oils registered by the United States National Board of Standards and Technology (GMIA, 2019) [6]. The formula used is as follows:

$$\eta = \frac{\eta^{o} \cdot d \cdot t}{d^{o}\, t^{o}}$$

Explanation:
η = viscosity of gelatin solution (milli poise)
η^{o} = viscosity of distilled water (milli poise)
d = density of gelatin solution (g/ml)
d^{o} = density of distilled water (g/ml)
t = efflux time of gelatin solution (seconds)
t^{o} = distilled water efflux time (seconds)

pH

A 0.2 g sample was weighed and dissolved in water 20 ml at 25°C. The sample was homogenized using a magnetic stirrer, and then the acidity degree was measured at room temperature with a pH meter (AOAC, 2019) [1].

Gel strength

Using distilled water, a 6.67% (w/v) gelatin solution was made. The solution was agitated with a magnetic stirrer until homogeneous, then heated to a temperature of 60°C for 15 minutes. Pour the solution into Standard Bloom Jars (bottles with a 58 – 60 mm diameter, 85 mm height), close, and let sit for 2 minutes. Incubate at 10°C for 16 – 18 hours. Next, it was measured using a TA-XT plus texture analyzer at a probe speed of 0.5 mm/second with a 4 mm depth (British Standard Institution /BSI 757, 1975) [4]. Gel strength is expressed in g bloom units.

Statistic analysis

The study approach was an experimental method with a completely randomized design, using five hydrochloric acid concentration treatments (1, 2, 3, 4, and 5%). Each treatment was repeated four times. The Treatment differences were tested using analysis of variance, and Duncan's Multiple Range Tests were used to compare differences between treatments,

Result and discussion

Rabbit skin gelatin viscosity

The hydrochloric acid effect in various concentrations on the rabbit skin gelatin viscosity can be seen in Table 1. Based on these findings, it is clear that the average viscosity value of rabbit skin gelatin treated with 1 - 5% hydrochloric acid from the lowest to the highest is 8.38 - 43, 95 mps. The hydrochloric acid concentration treatment of 1% (P1) and 2% (P2) complies with the gelatin viscosity quality standard according to GMIA (2019) [6], namely 15 – 75 mps.

Table 1: Various Concentrations Effect of Hydrochloric Acid on Viscosity, pH, and Gel Strength

Variables	Treatments				
	P_1	P_2	P_3	P_4	P_5
Viscosity (mps)	$43{,}96^{b}$	$16{,}18^{a}$	$10{,}72^{a}$	$8{,}38^{a}$	$8{,}56^{a}$
pH	$5{,}35^{a}$	$5{,}02^{a}$	$5{,}21^{a}$	$5{,}79^{a}$	$5{,}47^{a}$
Gel Strength (Gram Bloom)	$141{,}61^{b}$	$105{,}98^{b}$	$74{,}08^{b}$	$5{,}47^{a}$	$6{,}04^{a}$

Description: Superscripts in horizontal lines indicate substantial differences (P<0,05)

Viscosity value based on Table 1 shows that the concentration treatment from 1% (P1) to 4% (P4) decreased the viscosity value of rabbit skin gelatin and then increased again in the 5% concentration treatment (P5). This is thought to occur due to increased hydrochloric acid concentration used in the curing process. Acid can change triple helix collagen fibers into single chains (Junianto, *et al.* 2006) [13]. The curing agent breaks down the amino acid peptide binds into smaller molecular chains so that the viscosity decreases. In the 5% concentration treatment (P5) the viscosity value was higher than in the 4% concentration treatment (P4). This happened in accordance with the statement of Said, *et al.* (2011) [22], that although increasing the curing agent can reduce viscosity, viscosity can increase if the curing agent used can break peptide bonds at the right bonds with longer molecules.

The analysis of variance results shows that the hydrochloric acid addition at various concentrations has a significant effect (P<0.05) on the viscosity of gelatin. The 1% hydrochloric acid concentration treatment (P1) was significantly different (P<0.05) from the 2% - 5% concentration treatment (P2 – P5).

Treatment concentrations of 2% (P2), 3% (P3), 4% (P4), and 5% (P5) were not significantly different (P>0.05), presumably due to incomplete breakdown of collagen peptide bonds. According to Leiner (2006) [17], the longer the amino acid composition, the higher the gelatin viscosity value. This shows that increasing the concentration of hydrochloric acid used in the curing process causes the amino acid chains that should be long to become shorter, thereby reducing the viscosity value of gelatin. This is in line with Juliasti, *et al.* (2014) [12] statement that high concentrations of hydrochloric acid will cause the amino acid chains and their structures to open so that the chains become shorter and their viscosity decreases. As a result of the amino acid chains shortening, the relative molecular mass of the gelatin structure is also lower, and the viscosity is reduced (Mulyani, *et al.* 2012) [19]. Treatment with a 1% hydrochloric acid concentration is the best rabbit skin gelatin production.

pH of rabbit skin gelatin

The rabbit skin gelatin's average pH value treated with hydrochloric acid concentrations of 1% until 5% from the lowest to the highest is 5.02 – 5.79 (Table 1). The hydrochloric acid concentration treatment was 1% (P1), 2% (P2), 3% (P3), and 5% (P5) in accordance with the gelatin pH quality standard according to GMIA (2019) [6], namely 3.8 – 5.5. Based on BSI (1975) [4], the pH value allowed in gelatin products is 4.5 – 6.5 so gelatin made from rabbit skin can still meet these criteria.

The analysis of variance results indicated that the hydrochloric acid addition at various concentrations had no significant effect (P>0.05). This is thought to be influenced by the ability of the curing material to be absorbed into the collagen network. According to the Alfaro, *et al*. (2013) [2] statement that the extraction process can influence the pH value of gelatin. When swelling occurs due to the extraction process, the amino acid bond structure in the collagen molecule opens and little curing material or hydrochloric acid is trapped between the bonds so that the resulting pH value tends to be neutral.

The pH value was not significantly different (P>0.05) because the washing process had gone well so that not many H+ ions penetrated into the gelatin. Skin that have been cured with acids or bases previously undergo a neutralization and washing process before being processed further so that very few acid or base molecules are still bound to proteins. A pH value in the neutral range indicates that the raw materials washing or neutralizing process before entering the extraction process is running perfectly so that contamination of curing materials which can increase or decrease the pH significantly can be minimized (Said *et al.* 2011) [22].

Rabbit skin gelatin strength

The effect of various concentrations of hydrochloric acid on the rabbit skin gelatin gel strength can be seen in Table 1. Based on these findings, it is clear that the average strength value of rabbit skin gelatin gel with various concentrations of hydrochloric acid from the lowest to the highest is 5.46 - 141.61 grams. bloom. The hydrochloric acid concentration treatment of 1% (P1), 2% (P2), and 3% (P3) is in accordance with the gelatin strength quality standard according to GMIA (2019) [6], namely 50 – 300 grams Bloom.

The analysis of variance indicated that HCl concentration had a significant effect (P<0.05) on the strength of rabbit skin gelatin gel. It shows that there are differences in influence between treatments. Treatment with a hydrochloric acid concentration of 4% (P4) was not significantly different (P>0.05) from treatment with a hydrochloric acid concentration of 5% (P5). Meanwhile, treatments with concentrations of 1% (P1), 2% (P2), and 3% (P3) were not significantly different (P>0.05) from each other, but were significantly different (P<0.05) from the treatment hydrochloric acid concentration of 4% (P4) and 5% (P5).

This was thought to occur because the hydrochloric acid concentration treatment of 4% (P4) and 5% (P5) used a higher concentration of hydrochloric acid compared to the 1% (P1), 2% (P2), and 3% (P3) hydrochloric acid concentration treatment. The low value of gel strength can be influenced by the concentration of the curing agent and the time of the curing process because according to Sarabia, *et al.* (2000) [23] the higher the hydroxyproline content, the higher the gelatin gel strength produced, whereas if further hydrolysis occurs in the collagen that has become gelatin due to the high concentration of the curing agent and the long curing time it can cause shorter amino acid chains to form so that the gel strength is low. Short amino acid chains cause the interaction with water molecules to be lower so that they are unable to form a gel (Hafidz *et al*. 2011) [8].

The gel strength properties are influenced by the concentration of ingredients and curing time (Kolodziejska, *et al*. 2007) [16]. In this study, it was suspected that what influenced the gel strength was the concentration of the curing material because the curing time was carried out the same, namely 24 hours. The higher concentration of HCl will cause further hydrolysis of the collagen that has become gelatin and cause the peptide chains to shorten so that the gel strength is low. Longer peptide bonds can form aggregations to form more effective gel bonds, indicating high gel strength (Kaewdang *et al*. 2015) [14].

The gel strength is caused by the hydroxyproline content. Hydroxyproline is one of the gelatin amino acids together with proline and is one of the limiting amino acids in various proteins (Chaplin, 2005) [5]. Hydroxyproline causes hydrogen bonds between water molecules and free hydroxyl groups in gelatin to become more stable, thereby increasing gel strength (Sompie, *et al.* 2012) [26]. Gelatin molecules with short chains cannot form strong molecular bonds, especially hydrogen bonds or other weak bonds such as hydrophobic interactions and ionic interactions (Kittiphanattanabawon, *et al.* 2016) [15]. Treatment with a hydrochloric acid concentration of 1% is the optimum concentration in making rabbit skin gelatin.

References

1. [AOAC] Association of Official Analytical and Chemists. Official Methods of Analysis the 16th ed. Inc. Arlington. Virginia, 2019.
2. Alfaro, Alexandre, Trindade. *Physical and Chemical Properties of Wami Tilapia Skin Gelatin*. Food and Science Technology, 2013.
3. Astawan M, T Aviana. Pengaruh Jenis Larutan Perendaman Serta Metode Pengeringan Terhadap Sifat Fisik, Kimia, dan Fungsional Gelatin dari Kulit Ikan Cucut. Teknol. dan Ind. Pangan. 2003:14(1):7-13.
4. [BSI] British Standards Institution. Methods for Sampling and Testing Gelatin (Physical and Chemical Methods). BSI. London, 1975.
5. Chaplin M. *Gelatin*, 2005. [Online]. Available at: www.//Isbuc.ac.uk. (diakses pada Januari 2019).
6. [GMIA] Gelatin Manufacturers Institute of America. Gelatin Handbook. Gelatin Manufacturers Institute of America, 2019.
7. Gumilar J, A Pratama. Produksi dan Karakteristik Gelatin Halal Berbahan Dasar Usus Ayam. Jurnal Teknologi Industri Pertanian,2018:28(1):75-81.
8. Hafidz RMRN, Yaakob CM, Amin I, Noorfaizan A. Chemical And Functional Properties Of Bovine And Porcine Skin Gelatin. International Food Research Journal,2011:18:813-817.
9. Hasdar M, YD Rahmawati. Nilai pH, Titik Leleh dan Viskositas pada Gelatin Kulit Domba Asal Brebes yang Dikatalis Berbagai Konsentrasi NaOH. Jurnal Ilmiah Farmasi. 2016, 5(2).

10. Hastuti D, I Sumpe. Pengenalan dan Proses Pembuatan Gelatin. Jurnal Ilmu-Ilmu Pertanian, 2007, 3 (1).
11. Jannah A. Pembuatan Gelatin Halal dari Tulang Ikan Bandeng (Chanoschanos forskal) Sebagai Alternatif Pembuatan Gelatin Halal. Laporan Penelitian. Lemlit UIN Malang, 2007.
12. Juliasti R, AM Legowo YB Pramono. Pengaruh Konsentrasi Perendaman Asam Klorida pada Limbah Tulang Kaki Kambing terhadap Kekuatan Gel, Viskositas, Warna dan Kejernihan, Kadar Abu, dan Kadar Protein Gelatin. Jurnal Teknologi Hasil Pertanian. 2014, 7(1).
13. Junianto H Kiki, M Ine. Produksi Gelatin dari Tulang Ikan dan Pemanfaatannya Sebagai Bahan Dasar Pembuatan Cangkang Kapsul. Laporan Penelitian. Fakultas Perikanan dan Ilmu Kelautan Universitas Padjadjaran, 2006.
14. Kaewdang O, Benjakul S, Prodpran T, Kaewmanee, T, Kishimura H. Characteristics of Gelatin from Swim Bladder of Yellowfin Tuna (Thunnus albacores) as Influenced by Extracting Temperatures. Italia Journal Food Science,2015:27:366-374.
15. Kittiphanattanabawon P, Benjakul S, Sinthusamran S, Kishimura H. *Gelatin from Clown Featherback Skin: Extraction Condition*. *Food Science Technology*,2016:66:186-192.
16. Kolodziejska I, E Skierka, M Sadowska, W Kołodziejski, C Niecikowska. Effect of Extracting Time and Temperature on Yield of Gelatin from Different Fish Offal. Food Chem,2007:107:700-706.
17. Leiner PB. The Physical and Chemical Properties of Gelatin, 2006. [Online]. Available at: http:///www.pbgelatin.com. (diakses pada 23 Januari 2020).
18. Martianingsih N, L Amaja. Analisis Sifat Kimia, Fisik, dan Termal Gelatin dari Ekstraksi Kulit Ikan Pari (Himantura gerrardi) Melalui Variasi Jenis Larutan Asam. Prosiding Kimia FMIPA – ITS. Surabaya, 2010.
19. Mulyani T, Sudaryati, Rahmawati SF. *Hidrolisis Gelatin Tulang Ikan Kakap Menggunakan Larutan Asam.* Program Studi Teknologi Pangan, FTI UPN “Veteran” Jatim. Surabaya, 2012. [Online]. Available at: http://ejournal.upnjatim.ac.id/index.php/rekapangan/article/view/406. (diakses pada 22 Juli 2019).
20. Nurhalimah E. *Perbandingan Ekstraksi Gelatin Kulit Sapi Split dengan Proses Asam dan Basa*, 2010. [Online]. Available at: http://repository.ipb.ac.id/handle/123456789/61883. (diakses pada 14 Maret 2020).
21. Rapika, Zulfikar, Zumarni. Kualitas Fisik Gelatin Hasil Ekstraksi Kulit Sapi dengan Lama Perendaman dan Konsentrasi Asam Klorida (HCl) yang Berbeda. Jurnal Peternakan. 2016, 13(1).
22. Said MI, S Triatmodjo, Y Erwanto, A Fudholi. Karakteristik Gelatin Kulit Kambing yang Diproduksi Melalui Proses Asam dan Basa. 2011, 31(3).
23. Sarabia AI, Gomez-Guillen MC, Montero P. The Effect of Added Salts on the Viscoelastic Properties of Fish Skin Gelatin,2000:70(1):71-76.
24. Sasmitaloka KS, Miskiyah, Juniawati. Kajian Potensi Kulit Sapi Kering Sebagai Bahan Dasar Produksi Gelatin Halal. Buletin Peternakan,2017:41(3):328-337.
25. Schrieber R, Garies H. Gelatin Handbook: Theory and Industrial Practice. Wiley-VCH, 2007.
26. Sompie M, S Triatmojo, A Pertiwiningrum, Y Pranoto. Pengaruh Umur Potong dan Konsentrasi Larutan Asam Asetat terhadap Sifat Fisik dan Kimia Gelatin Kulit Babi,2012:10(1):15-22.
27. Youlanda H. Ekstraksi dan Evaluasi Gelatin dari Kulit Sapi Yang Telah Mengalami Proses Buang Bulu Menggunakan Hidrolisis Asam. UIN Syarif Hidayatullah. Jakarta, 2016.
28. Zhang J, Duan R, Ye C, Konno K. Isolation and Characterization of Collagens from Scale of Silver Carp (Hypophthalmichys molitrix). Journal of Food Biochemistry,2010:34:1343-1354