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The Effect of HCL Concentration on Gelatin Yield, Moisture, and ASH Content from Rabbit's (*Lepus Nigri Collis*) Skin

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Abstract
Rabbits are slaughtered to get meat, while local rabbit skins are not used because the quality of the skin is poor, therefore local rabbit skins become waste. Rabbit skin contains collagen which is a potential source of gelatin. Gelatin can be obtained from the denaturation of collagen in rabbit skin. HCl is a strong acid with many hydrogen ions so it can hydrolyze collagen fibers into gelatin. This research aimed to examine the influence of HCl concentrations on yield, moisture content, and ash content in gelatin from rabbit skin. This study uses an experimental method with a Completely Randomized Design followed by Tukey's test. There were five concentrations of HCl (T_1=1%, T_2=2%, T_3=3%, T_4=4% and T_5=5%) with three replications. Statistical analysis showed that HCl treatment had a significant effect ($P < 0,05$) on yield, water content, and ash content in rabbit skin gelatin. The best gelatin in the treatment is 1% HCl which produces gelatin with 3,02% yield, 14,10% moisture content, and 1,32% ash content.

Keywords: gelatin, rabbit's skin, HCl, yield, moisture, ash content

Introduction

Gelatin is a protein generated from the partial hydrolysis of collagen taken from animals' bones, connective tissue, and skin (hide) (Feng et al, 2022). Local Indonesian rabbit (*Lepus nigricollis*) skin can be used as raw material for gelatin because it contains a lot of collagen and is not widely used. Gelatin has various uses in the medicine, food, photography, and cosmetics industries (Onouma, et al., 2015). Gelatin is commonly used as an ingredient to increase thickness, elasticity, and emulsification (Lin et al., 2017).

Gelatin is classified into two categories based on differences in the production process, namely type A and type B. Making Type A gelatin is treated with immersion in an acid solution so this process is known as the acid process while making type B gelatin is a base treatment called the alkaline process. Making gelatin by acid soaking is able to convert triple helix collagen fibers into single strands, whereas alkaline soaking solutions are only capable of producing double strands. Hydrochloric acid is the most appropriate type of acid to use in the extraction process because the price of hydrochloric acid is more economical, it is widely available, has less ash residue (because its molecular weight is lower), and is less corrosive compared to sulfuric acid. The time required for the acid process is generally 2-48 hours, much faster than the base process, which is around 3 months (Schrieber and Garies, 2007).

Assessment of the quality of gelatin used in various types of industry is influenced by physical properties such as water content and ash content. Apart from that, one of the parameters that influences the efficiency of making gelatin is yield. The quantity of collagen transformed into gelatin determines the increase in yield. Using strong acids increases the concentration of H+ ions in the curing solution, accelerating the hydrolysis process. The higher the hydrolysis rate, the more collagen is converted into gelatin, increasing the yield value. If the hydrolysis rate is greater, the breakdown of the triple helix into α, β, and γ chains will also be greater, causing more gelatin conversion (Ilona, et al., 2007). Research can be said to be effective if it produces a high yield value so that the treatment applied is more efficient (Santoso, 2001).

A material's water content is the proportion of water bound in its oven-dry weight. The higher the hydrochloric acid content in the solution, the lower the water content of the gelatin produced, and the longer the skin is soaked, the lower the gelatin's water content produced. As the acid content increases, the soaking solution contains more H^+ ions. This causes more collagen to be hydrolyzed, the number of hydrophobic groups increases on the surface of the protein molecule and the size of the molecule decreases, thereby reducing the water storage capacity (Hazmi, et al., 2017). Water content in food impacts the freshness, taste, texture, acceptability, appearance, and quality of food ingredients as well as their durability (Winarno, 2002). The gelatin quality standard based on SNI (1995) is 16% maximum for moisture content.

Ash content shows the amount of inorganic material contained in organic material. The higher the acid concentration, the lower the ash content produced, this is possible because there are more hydrogen ions [H+] contained in the solution. This means that the ability to dissolve inorganic substances in the skin is also higher. So that at a high concentration the residual ash contained in the gelatin will be low. The requirements for gelatin according to SNI (06-3735-1995) is a maximum of 3.25% for ash content.

The best research on cowhide gelatin was at an HCl concentration of 3% with a soaking time of 12 hours resulting in a high yield value of 37.93% (Rapika, et al., 2016). Research on gelatin from the best curing sheepskin shaving process waste was carried out 24 hours with an HCl concentration of 3% resulting in a yield of 7.48% and a water content of 10.9% (Gumilar, et al., 2019). Another research, namely research on yellowfin tuna skin gelatin using 3% HCl conducted by Dayva, et al., (2018) produced a water content value of 1.75% and an ash content of 1.51%.

Several studies on gelatin production using HCl in the demineralization stage are the best gelatin production from cowhide using an HCl concentration of 3% with a soaking time of 12 hours, which can produce gelatin with a high yield value, namely 37.93% (Rapika, et al., 2016). Gelatin production from the waste of sheepskin pickle from the shaving process can use 3% HCl in the demineralization process for 24 hours produce the best gelatin with a yield of 7.48% and a moisture content of 10.9% (Gumilar, et al., 2019). Another research, on yellowfin tuna skin gelatin, using 3% HCl, produced gelatin with a moisture content value of 1.75% and an ash content of 1.51% (Dayva, et al., 2018). There is not much information about research on the production of gelatin from rabbit skin using HCl in the demineralization process, therefore the aim of this research is to determine the best use of HCl for the quality of rabbit skin gelatin in terms of yield, moisture content, and ash content variables.

Materials And Methods

Material

Rabbit skin was obtained from local farmers in Lembang, Bandung district, West Java province, Indonesia. Hydrochloric acid (Merck, Germany). water bath (Julabo, Germany), drying oven (Julabo, Germany), furnace (Thermo Fisher Scientific, USA), analytical balance (Ohaus, USA).

Method

Gelatin Production

The method for gelatin production is based on the method of Said, et al. (2011) and Gumilar et al., (2018) with slight modifications. The procedure for producing rabbit skin gelatin is as follows: The rabbit skin is soaked in a 6% lime solution of the skin weight, then stirred periodically so that the lime does not settle and enter the skin. The water used should be cold, and the skin soak should be kept at a low temperature for 1-2 days until the hair is easy to remove (dehairing). Skin that has been clean of hair is washed with water for 20-30 minutes until the skin pH is neutral (6-7) and clean of adhering dirt. Rabbit skin is soaked in water at a temperature of 90 ± 3^{o}C for 15 minutes to remove remaining fat on the skin (degreasing) then cut into small pieces (the size is 3 cm X 3 cm) then weighed with each unit weighing 150 grams/unit and put into a beaker glass. Rabbit skin is added with 1%, 2%, 3%, 4%, and 5% HCl solution (demineralization) then covered with aluminum foil and soaked for 24 hours to remove calcium and mineral salts. Rabbit skin becomes soft which is called ossein. This ossein contains collagen and protein. The ossein is then washed with distilled water until the pH is neutral (6-7). Next is the extraction stage, the ossein is then put into a beaker glass and distilled water is added. The ossein was then placed in a water bath to be extracted at a temperature of 80^{o}C for 7 hours. The extraction products were filtered, evaporated, and then dried in a drying oven at 55^{o}C for 52 hours.

Yield

The yield calculation method uses the AOAC method (2019) with the formula:

$$\text{Yield (\%)} = \frac{\text{Gelatin weight}}{\text{Rabbit skin weight}} \times 100\%$$

Moisture content

Determination of moisture content refers to AOAC, 2019 with the formula used to calculate water content as follows:

$$\text{Moisture content (\%)} = \frac{(W1-W2)}{(W1-W0)} \times 100\%$$

Ash Content

Determination of ash content (AOAC, 2019) by drying gelatin in an oven at 600^{o}C for 3-5 hours. After that, cool it in a desiccator, weigh the ashes, and calculate with the following formula:

$$\text{Ash conte (\%)} = \frac{\text{Ash weight}}{\text{Gelatin weight}} \times 100\%$$

Result And Discussion

Effect of HCl Concentration Treatment on Yield

Yield is the dry gelatin weight ratio to the fresh rawhide weight. It is very important to calculate the yield to determine the level of effectiveness and efficiency in making rabbit skin gelatin. The higher the yield percentage, the more effective and efficient the research carried out will be. Rabbit skin gelatin with various HCl concentrations in the demineralization process produces gelatin yield percentages that ilustrated in Table 1.

Table 1. Effect of Various HCl Concentrations on the Yield, Moisture Content, and Ash Content of Rabbit Skin Gelatin

Variables	Treatments				
	T_1	T_2	T_3	T_4	T_5
Yield (%)	3,02^{c}	2,13^{b}	0,37^{a}	0,11^{a}	0,34^{a}
Moisture content (%)	14,10^{a}	14,73^{a}	14,53^{a}	17,39^{bc}	18,03^{c}
Ash content (%)	1,32^{a}	1,52^{a}	3,67^{a}	11,96^{b}	8,95^{c}

Note: Different superscripts on the same horizontal row indicate significantly different values

Table 1 shows that gelatin with 1% HCl treatment, namely 3.02%, has the highest gelatin yield, then 2% HCl treatment, namely 2.13%, 3% HCl treatment, namely 0.37%, 5% HCl treatment, namely 0.34% and the lowest yield was gelatin with a 4% HCl concentration, namely 0.11%. Variance analysis revealed a significant effect ($P<0.05$) of the treatment using HCl various concentrations on the yield. The average yield generated by 3% HCl concentration (T3) was not significantly different from the yield produced by 4% HCl concentration (T4) and 5% HCl (T5) but was considerably different ($P<0.05$) by treatment concentration of 1% HCl (T1) and 2% HCl (T2). T1 had the highest significant difference ($P<0,05$) compared to other treatments.

According to Gumilar, et al (2019), the high concentration of HCl used as a soaker can lower the yield of gelatin, this can be caused by the higher the concentration of HCl, the stronger the acid level of the solution used and can hydrolyze the collagen structure, the triple helix does not just become a chain. single but until it is completely hydrolyzed into its composition of amino acids, causing the loss of a certain amount of collagen when washing ossein. The yield is closely connected to the transformation of collagen into gelatin (Gumilar et al., 2023)

Dayva, et al (2018), found that the differences in the concentration of the HCl solution influence the gelatin-produced yield. This is because the concentration of hydrochloric acid (HCl) increases, the collagen structure becomes more open and more collagen will be hydrolyzed so that more gelatin will be extracted. The results of the research on the yield of rabbit skin gelatin with an HCl concentration of 1% were 3.02%, which was greater than the research of Endang, et al (2018) on the yield of Belinda fish bone gelatin with an HCl concentration of 1%, namely 0.57%.

Effect of HCl Concentration Treatment on Moisture Content

The moisture content test result of rabbit skin gelatin presented in Table 1 shows that the greatest moisture content value was at 5% HCl concentration (T5), namely 18.03%, followed by 4% HCl treatment (T5) at 17.39%, then 2% HCl (T2) namely 14.73%, HCl 3% (T3) namely 14.53%, and the lowest treatment with HCl 1% (P1) namely 14.10%. T1 until T4 comply with the maximum gelatin moisture content requirements according to the Indonesian National Standard (SNI, 1995), namely 16%, while T4 and T5 exceed the SNI provisions but still comply with the quality standards according to JECFA (2003) that maximum gelatin moisture content is 18%.

Analysis of variance calculations to astimate the effect of treatment on moisture content revealed that the use of various concentrations of hydrochloric acid had a substantial effect on the moisture content of rabbit skin gelatin ($P<0.05$). Data from Tukey's test results in Table 1 states that the concentration of 1% HCl (T1) is not significantly different ($P>0.05$) from the 2% HCl (T2) and 3% HCl (T3) treatments but is significantly different ($P<0.05$) lower than 4% HCl (T4) and 5% HCl (T5) treatments. The 4% HCl concentration treatment (T4) was not significantly different ($P>0.05$) from the 5% HCl concentration treatment (T5).

The moisture content increases as the concentration of HCl increases. The high gelatin's moisture content is possible due to the large number of hydrogen bonds that bind the gelatin structure, so that water will be mobilized within the gelatin structure. This makes the gelatin's water binding capacity to be very strong, resulting in a high moisture content in gelatin (Nazilatun, 2017). According to Sebastian (2014), type A gelatin with high acid treatment has an isoionic region. The isoionic point is when a protein treated with acid forms zwitter molecules, causing the protein to coagulate. In this condition, the water bound in protein molecules will be more difficult to evaporate. According to See, et al (2010), this varying water content is influenced by the length of drying time, humidity during storage, and water permeability of the storage container.

Effect of HCl Concentration Treatment on Ash Content

The data presented in Table 1 shows the results of varying average gelatin ash content. The quality requirement for gelatin ash content referred to the Indonesian National Standard (SNI, 1995) is a maximum of 3.15%, so in this study, the treatment concentration of 1% HCl and 2% HCl met the gelatin quality standards, namely 1.32% and 1.52%, while HCl was 3%, HCl 4%, and 5% HCl exceeds the gelatin quality standard limit in Indonesia.

The variance analysis calculation results indicated that varying the HCl concentration had a significant effect ($P<0.05$) on the ash content of the gelatin produced. 1% HCl concentration (T1) was not significantly different ($P>0.05$) from 2% HCl treatment (T2) and 3% HCl (T3) but was significantly ($P<0.05$) lower than 4% HCl treatment (T4) and 5% HCl (T5). According to Sebastian (2014), at high HCl concentrations, amphoteric proteins will form zwitter molecules and clump together. This process can bind mineral molecules and be carried away during the extraction and drying process so that the resulting ash is high. According to Suryati, et al (2015), the mineral composition of the raw material, influence the high proportion of gelatin ash produced, as do the filtration and hydrolysis techniques used.

Hydrochloric acid (HCl) is an inorganic acid so that the substances contained in rabbit skin can decompose and form inorganic minerals during demineralization and extraction at high temperatures so that inorganic substances still stick to the raw material and are carried away during the ashing process. The high concentration of HCl can change the collagen arrangement from triple helix to single chain so that the resulting ossein is softer, broken, and carried away during filtration. This is consistent with Astawan, et al (2002) remark that incomplete filtration causes a large amount of ossein powder to be transported in the gelatin filtrate. When the gelatin is transformed into gel, the fine ossein powder goes through the filter and forms a precipitate. The high difference in ash content from gelatin ash content between treatments is caused by the presence of mineral components that are still attached to collagen and have not been released during the washing process, thus they are extracted and carried away during the washing process.

Conclusion

The HCl use with different concentrations affects the yield, water content, and ash content of the rabbit skin gelatin produced. The best rabbit skin gelatin is found in the 1% HCl (T1) concentration treatment which produces a yield of 3.02%, a moisture content of 14.10% and an ash content of 1.32% so that it meets gelatin quality standards in Indonesia.

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