Brebes Sheep Skin That is Hydrolyzed With Excess Acid Solution (CH_3COOH) and Sitrate Acid ($C_6H_8O_7$) Became Gelatin

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Abstract

Local resources from Brebes Regency that have not been maximized properly are sheep skin. Brebes sheep skin can be converted into gelatin. This study aims to determine the quality of the yield and gelatin protein of sheep skin hydrolyzed using weak acids. The main ingredient of this research is sheep skin from Brebes Regency, which is 1-2 years old. The research method uses a completely randomized design (CRD) 2 x 3 factorial pattern where the first factor is the soaking material (CH_3COOH 2% v / v and $C_6H_8O_7$ 2% v / v) and the second factor is the immersion time (2 hours, 3 hours and 4 hour), then proceed with the Real Difference test using the Duncan Multiple Range Test (DMRT). The yield measurement results showed the percentage of sheep skin gelatin is 10,12-10,77%, and the measurement of sheep skin gelatin protein showed a percentage of 70,96-72,87%. The ability of CH_3COOH 2% in hydrolyzing sheep skin collagen is better than $C_6H_8O_7$ 2%. The highest percentage of yield and protein is at 4 hours soaking time for each type of solution.

Keywords: Gelatin, Low Acid, protein, sheep skin, rendement

Introduction

Brebes sheep skin is one of the local potentials whose utilization has not been maximized by the community, government or the private sector. The economic value of Brebes sheep skin can be increased by processing it into gelatin. Gelatin is an organic biopolymer with the main component of structural protein in the form of collagen. Gelatin is obtained through the process of collagen hydrolysis from the skin with the help of catalyst chemicals, namely acidic or alkaline solutions. Hydrolyzed gelatin using acid catalysts will produce type A gelatin, whereas gelatin hydrolyzed using an alkaline solution catalyst will produce type B. Gelatin hydrolyzed gelatin with acid solution is more likely to be applied to the food field (Kittiphattanabawon et al., 2016), whereas gelatin which is hydrolyzed with acid solution is more likely to be applied to the food sector (Kittiphattanabawon et al., 2016), whereas gelatin which is hydrolyzed hydrolysis with an alkaline solution is more suitable for non-food products because it has a high pH.

The ability of acid solution in helping the hydrolysis process of skin collagen is also not the same depending on the type of acid solution, concentration and soaking time. The types of acids commonly used for food products are CH_3COOH (acetic acid) and $C_6H_8O_7$ (citric acid). In the community acetic acid is commonly called vinegar and citric acid commonly used as additives regulating acidity in food products. Research on hydrolyzing animal skin collagen using CH_3COOH has been carried out by Said et al., (2011) with a fairly long immersion treatment. Hashar and Rahmawati (2017) has

catalyzed Brebes sheep skin research with catalyst NaOH solution. Acid solution is able to break the collagen peptide chain into a single chain and can improve the quality of collagen proteins, in contrast to alkaline solutions which are only able to break collagen solutions into double chains. Besides that, soaking with acid solution is faster in swelling of the skin and hydrolysis results with more acidic laturan. Therefore this study aims to determine the quality of yield and gelatin protein of sheep skin hydrolyzed using weak acids CH_3COOH and $C_6H_8O_7$.

Materials and Methods

Materials

The research was conducted in the integrated laboratory of Muhadi Setiabudi University, Brebes. The main ingredient of this research is sheep skin from Brebes Regency, aged 1-2 years. The chemicals are used a solution of acetic acid and citric acid with each concentration of 2% (v / v). Other materials are used aquades and water. Equipment used in the gelatin production process are analytical scales, filter paper, hot plates, thermometers, measuring cups, beaker cups, petri dishes, measuring flasks, erlenmeyers, refrigators, and ovens.

Methods

Fresh sheep skin that is obtained from breeders and slaughterhouses in Brebes Regency is separated from leather and wool using sharp razor blade. The selected sheep skin is leather that is not suitable for tanning or low quality leather due to an unprofessional hindrance process. This low quality leather has a cheap price. The next process is removal of the remnants of meat or fat that is still attached to the skin by using a sharp knife. Then then washed with clean water. Skin that has been cleared of wool and the remnants of meat / fat drained so that water does not leave a lot of skin. Then do the weighing of fresh skin and then in the fresh skin is reduced in size to ± 2 x 2 cm. Then immersed in a solution of CH_3COOH 2% (v / v) and $C_6H_8O_7$ 2% (v / v) with soaking time for 2 hours, 3 hours and 4 hours. The results of soaking the sheep skin with a chemical solution, then washed with water until clean and reached ± pH 7. Then extracted using a hot treatment method with a temperature of 50 - 55^oC which is done in stages for 4 hours, 3 hours, and 2 hours. Furthermore, the drying process follows the process carried out by Hasdar and Rahmawati (2016).

Research parameter

The parameters of this research are: rendemen and protein. Rendemen is obtained from the ratio of the dry weight of the resulting gelatin to the weight of the extracted chicken leg skin multiplied by 100%. Protein was measured by the Kjeldahl method. Protein content in the sample by multiplying the N produced by the multiplier factor 5.55. This method is based on the oxidation of the nitrogen gelatin component with sulfuric acid, so ammonium sulfate is obtained. After the solution is made alkaline with NaOH, ammonium is distilled and captured with boric acid to form salts. Determining the amount of ammonium that is distilled is carried out by titration of salt formed with HCl. Nitrogen percentage and crude protein content are calculated using the formula:

$$\text{Nitrogen} = \frac{[(\text{ml HCl} - \text{ml blangko}) \text{x N HCl x } 14.007)]}{\text{the weight of dry sample (mg)}} \; x \; 100\%$$

Crude Protein Levels = % N x 5,55

Data analysis

This research uses a Completely Randomized Design (CRD) 2 x 3 Factorial pattern where the first factors are 2% (v / v) CH_3COOH and 2% (v / v) $C_3H_8O_7$. The second factor is the duration of immersion (2 hours, 3 hours and 4 hours). The analysis of this research uses the multiple-analysis method. All data obtained were then analyzed using the One-Way ANOVA factorial pattern method using SPSS 23.0 Statistics Software. The significant level is set at $\alpha = 0.05$. If there are significant differences between the treatments, followed by a Real Difference test using the Duncan Multiple Range Test (DMRT).

Results and Discussion

Rendemen

Rendemen Gelatin is defined as a product resulting from the skin extraction process in a dry and clean form expressed as a percentage. The percentage of rendemen gelatin shows the final result of the effectiveness of chemical runoff in the hydrolysis process of sheep skin. The greater the percentage of gelatin yield shows the more effective the chemical solution used. The amount of rendemen gelatin is directly proportional to the high percentage of sea protein contained in the gelatin.

Rendemen Gelatin of Brebes sheep skin hydrolyzed using a weak acid solution is presented in table 1 below:

Table 1. Average of Sheep Skin Rendemen Gelatin in Hydrolyzed Sheep Skin Using Weak Acid Solutions.

Types of Acid solutions	Long Soaking	Rendemen (%)
CH_3COOH 2%	2 hour	10.67 ± 0.62^{a}
	3 hour	10.73 ± 0.82^{a}
	4 hour	10.77 ± 0.52^{a}
$C_6H_8O_7$ 2%	2 hour	10.12 ± 1.22^{b}
	3 hour	10.35 ± 0.61^{b}
	4 hour	10.37 ± 0.55^{b}

Note: ^{abc} different superscript letters in different rows / columns indicate a significant difference ($P < 0.05$)

Based on the results of the statistical tests in Table 1 show that the different types of solutions have a very significant effect (P <0.01), this phenomenon occurs due to the main nature of the chemical solution used as a sheep skin marinade.

CH_3COOH 2% solution is able to break the skin peptide bonds better than $C_6H_8O_7$ 2% solution. So that the yield of sheep skin gelatin which is catalyzed using 2% CH_3COOH solution is higher. High or low rendemen of gelatin produced is greatly influenced by the process of treatment of skin collagen proteins (Rakhmanova et al., 2018). The treatment process in question is the selection of catalyst solution, con catalyst solution, duration of immersion with catalyst solution (Hasdar and Rahmawati, 2017).

Based on Table 1. It can be seen that the highest yield is 10.77%, found in the 2% CH_3COOH treatment with 4 hours soaking time. Further testing of the Duncan method showed that the difference in the length of time soaking sheep skin was not significantly different. This shows the ability of 2% CH_3COOH solution and 2% $C_6H_8O_7$ with a short susceptibility does not have a significant impact. This means that the ability of a 2% CH_3COOH solution and 2% $C_6H_8O_7$ to break the sheep's tropokolagen protein chain into a single chain in a short susceptible period does not increase the yield of collagen extracts. Long time-sensitive to skin immersion with acid solution that will make the collagen structure more open so that it facilitates the process of protein solubility when extracted in hot water (Boran et al., 2010)

The Protein Sheep Skin Gelatin

Gelatin is the main structural protein found in the skin. So that the measurement of gelatin quality has two objectives, namely the purpose of identifying gelatain raw materials and the purpose of using gelatin for advanced products. Gelatin as a type of conversion protein that is produced through the process of collagen hydrolysis, basically has high protein content.

Measurement of protein quality for the purpose of using as raw material for advanced products is very important, because it will be closely related to the strength of the gel contained in gelatin. The average protein content can be seen in table 2 below:

Table 2. Average Protein Level (%) of Sheep Skin Gelatin Hydrolyzed with Weak Acid Solutions

Types of Acid solutions	Long Soaking	Up Protein (%)
CH_3COOH 2%	2 hour	72.57 ± 2.06^{a}
	3 hour	72.74 ± 0.27^{a}
	4 hour	72.87 ± 1.74^{a}
$C_6H_8O_7$ 2%	2 hour	70.96 ± 0.79^{b}
	3 hour	70.98 ± 0.27^{b}
	4 hour	70.96 ± 0.52^{b}

Note: ^{abc} different superscript letters in different rows / columns indicate a significant difference (P <0.05)

The highest protein content was found in sheep skin gelatin which was soaked with CH_3COOH 2% for 4 hours, namely 72.87%, while the lowest was in sheep skin gelatin $C_6H_8O_7$ hydrolysis 2% for 2 hours and 4 hours, 70.96%. Long time immersion in sheep skin in this study did not affect the quality of the protein produced. The protein quality in this study is directly proportional to the yield produced.

Based on the results of Duncan's further tests showed no difference in producing protein based on the length of immersion. This means that it takes vulnerable more time between treatments to see differences in protein quality. This is in line with research by Tkaczewska et al. (2018) which states that the time of skin soaking can affect the quality of the resulting gelatin. Chemical solutions require time to break the polymer chains of amino acids in the skin (Hassan et al., 2018).

Based on Table 2. the quality of sheep skin gelatin protein is different between those produced by 2% CH_3COOH and 2% $C_6H_8O_7$. This shows that in the process of loosening the helical triple bonds of collagen protein in sheep skin the ability of CH_3COOH 2% is better when compared with 2% $C_6H_8O_7$. High levels of gelatin protein can also be caused by the state of the raw material. The process of handling raw materials and storing and preserving leather raw materials also affect the quality of the skin gelatin produced (Sinthusamran et al., 2014). In this research, raw materials used in sheep skin are fresh and not damaged so that the resulting protein content of sheep skin gelatin is quite high.

Conclusion

The ability of a 2% CH_3COOH solution is better when compared with a 2% $C_6H_8O_7$ solution in producing high rendemen of gelatin and protein. Soaking time of 2 hours, 3 hours and 4 hours did not have an effect or difference in producing gelatin yield and gelatin protein. The highest rendemen and sheep gelatin protein in this research were 10.77% and 72.87%, resulting in a 2% CH_3COOH solution with 4 hours soaking time.

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