Molecular Weight Distribution, Amino Acid and Functional Groups Profile of Gelatin Bovine Split Hide as Influenced by Acid Pre-Treatment

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Abstract
Gelatin is natural substance obtained from the partial hydrolysis of collagen from the skin, bones and connective tissue. This Study aims to weight molecular, amino acids and functional groups profile of gelatin from bovine split hide using a variation of acetic acid concentration as a curing solution. The materials were gelatin from split bovine hide or hide split of flesh part from tanning industry. The materials were divided into three treatments T1, T2 and T3 (curing in 0.1M; 0.3M; 0.5M acetate acid) for 24 hours, then being extracted graded at 60oC, 70oC, 80oC each for 5 hours. Electrophoresis result showed the distribution molecular weight between 25-40 kDa. Amino acid profile of gelatin was similar to the amino acid composition of collagen with a high level of hydroxyproline. The intensity of the absorption of infrared (FTIR) consisted of functional groups O-H, C=O, C=C, C-H, and C-O. The result of amino acid and functional groups profile gelatin from bovine split hide by curing acetate acid concentration of 0.5 M provided the best characteristics of gelatin.

Keywords: characteristic; gelatin; bovine split hide; acetic acid

INTRODUCTION
Gelatin is a natural substance obtained from the partial hydrolysis of collagen. Industries utilized gelatin as a raw material included food industry, pharmaceutical, healthcare, photography, cosmetics and engineering. This means that demand of gelatin consumption is very high. Demand gelatin in Indonesia in 2014 reached became 3.966.254 kg [1].

The high demand for gelatin is not matched with local gelatin production both in terms of quality and most of the gelatin used in Indonesia is imported. Imported gelatin can be made of animal skin and bones, such as cows, fish, or pork. Gelatin derived from pig skin and bones may harm consumers, especially for Muslim community. The raw material for the manufacture of gelatin any much. One potential used as raw materials are bovine split hide, a byproduct of the tanning industry.

Tanning is processing raw hides to be tanned leather. During the process one of the important stage is splitted the hide to get the expected thickness of leather. Then, the unexpected leather was called splitted hide which still contains the collagen protein. Collagen which is hydrolyzed can produce gelatin [2,3].
Curing is one of important process in the production of gelatin. At this stage of curing, acid or base solution can cause hydrolysis in the form of chemical bonding of the polymer of collagen proteins into simple molecules (monomers). Gelatin with acid curing process is better than the base curing process because the acid is able to change the triple helix collagen fibers into a single chain, in short time and cheaper costs [4]. The coincided results have been reported of other researcher [5], that the shorter curing time of making of gelatin using acid curing process, showed the higher gel strength, viscosity and clearer color.

Application of the high concentrations of curing agent is possible to increase the quantity of gelatin products, because more molecules are collagen hydrolyzed. In terms of quality, the application of high concentration curing agent may not guarantee better results.

The Treatment acetic acid concentration probably affect the amount of gelatin extraction [6]. Acetic acid concentration of 0.2 M influenced the characteristic of the unicorn leatherjacket skin gelatin [7]. Research by treatment with acetic acid for extraction have been conducted regarding pig skin gelatin [8], goat skin gelatin [9], fish skin gelatin [10,11,12] and chicken legs skin gelatin [13]. This Study aims to weight molecular, amino acids and functional groups profile of gelatin from bovine split hide using variation of acetic acid concentration as curing solution.

MATERIALS AND METHODS

Materials and Equipment
Bovine split hide was obtained from Magetan Tannery Industry, East Java, Indonesia. Chemicals used for curing is acetic acid (CH3COOH) 0.1 M; 0.3 M, 0.5 M, dekaltal and distilled water. The equipment used is Water bath (Memmert Type WNB &-45), Oven (Memmert), analytical scale (Sartorius TE 2145), volumetric flash, beaker, erlenmeyer, thermometer, funnel cups, measuring pipette, blender, knife, equipment testing: HPLC (Shimadzu LC10), Spectrofotometer (Shimadzu PC-8201), Elektrophoresis (ATTO pugeran AE 6531) dan FTIR (Perkin elmer. Inc, USA).

Preparation of gelatin
Bovine split hide pieces were fleshed in dry condition and soaked in water for 2 days then delimed with decaltal 1% for 30 minutes. The delimed pieces were neutralized in running water to a pH 6.0 – 7.0. The hide was cut into small pieces to the size of 1-2 cm² and weighed. Gelatin was prepared by type A (acid) method. The bovine split hide was soaked at different concentration of acetic acid solution 0.1 M; 0.3 M; 0.5 M for 24 hours. After soaked, sample were neutralized with water to pH 5.0-6.0. The extraction process was performed on three stages at a temperature of 60°C, 70°C and 80°C (each stage for 5 hours). Filtrate obtained by filtering with filter paper no 0.45µm. In Order to increase the concentration of gelatin the solution was water bath exposed at 60°C for 5 hours. Pouring in the tray, and dried in an oven at 50°C for 36- 48 hours. Gelatin sheet were milled and packaged in plastic bottles and stored in a desiccator for subsequent process [2].

Methods of Analysis

Distribution of Molecular Weight
The molecular weight distribution is determined according to the method [14] with Sodium Sulfate Dedocyl Polyacrilamide gel electrophoresis (SDS PAGE).

Amino Acid Profile
Amino acid profile of the samples was determined by using of the High Performance Liquid Chromatography (HPLC) method [15].

Functional Group Profile
Analysis functional group using 96600 Frontier FT IR (Perkin elmer. Inc, USA) equipped with universal Attenuated Total Reflectance (ATR).
Experimental Design and Data Analysis
The study was conducted with experimental. The data obtained is displayed in the form of tables and figures. Display data obtained were analyzed descriptively, further qualitative data to explain the pattern of relationships and similarities with commercial gelatin or reference.

RESULT AND DISCUSSION
Distribution of Molecular Weight
Results gelatin gel electrophoresis analysis of bovine split hide is presented in Figure 1. Figure 1 indicates that the pattern of protein molecules gelatin from bovine split hide by acetic acid curing so obvious, especially for sample T1 (curing in 0.1M acetic acid) for T2 and T3 seen some faintly visible bands. Whereas, the commercial gelatin (Gk) seems more obvious. The molecular weight of gelatin for T2 and T3 in the range of 25-40 KDa. T1 cannot reveal the band pattern of protein molecules. It caused concentration of 0.1 M acetate acid has not been cut off polypeptide chain so that the polipetida chain has not been able to passed elekrophoresis gel. Distribution of molecular weight of gelatin was closely related to long-chain amino acids bond and gel strength levels [16].

Amino Acid Profile
Table 1 presented the amino acid composition of bovine split hide gelatin. The affect of concentration curing agent gives the percentage composition of the different amino acids each treatment. The performance of acid curing allows some specific amino acids undergo denaturation and leaching process so finally it was permanently damaged and effected to change in the amino acid composition [17]. An amino acid a major component of collagen protein constituent was very sensitive to the activity of an acid. Some amino acids can be damaged by the activity of acid substances [18].

<table>
<thead>
<tr>
<th>No</th>
<th>Amino acid</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>CG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspartic acid</td>
<td>3.4</td>
<td>3.2</td>
<td>3.9</td>
<td>4.6*</td>
</tr>
<tr>
<td>2</td>
<td>Glutamic acid</td>
<td>6.6</td>
<td>6.1</td>
<td>7.5</td>
<td>7.4*</td>
</tr>
<tr>
<td>3</td>
<td>Serine</td>
<td>2.3</td>
<td>2.3</td>
<td>2.8</td>
<td>3.9*</td>
</tr>
<tr>
<td>4</td>
<td>Histidine</td>
<td>1.2</td>
<td>1.2</td>
<td>1.3</td>
<td>0.4*</td>
</tr>
<tr>
<td>5</td>
<td>Glysine</td>
<td>10.1</td>
<td>10.1</td>
<td>11.3</td>
<td>34.2*</td>
</tr>
<tr>
<td>6</td>
<td>Arginine</td>
<td>5.2</td>
<td>4.6</td>
<td>6.2</td>
<td>4.7*</td>
</tr>
<tr>
<td>7</td>
<td>Alanine</td>
<td>5.6</td>
<td>5.6</td>
<td>6.9</td>
<td>11.3*</td>
</tr>
<tr>
<td>No</td>
<td>Wave number at the peak absorption (cm$^{-1}$)</td>
<td>Estimate of functional groups</td>
<td>Name of functional groups</td>
<td>Wave number at the peak absorption (cm$^{-1}$)</td>
<td>Estimate of functional groups</td>
</tr>
<tr>
<td>----</td>
<td>---------------------------------------------</td>
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<td>---------------------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>T1 (Acetat acid 0.1M)</td>
<td>3268.91 O-H Carboxylic acid</td>
<td>1 2969.91 O - H Carboxcylic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 2</td>
<td>1630.60 C=O Amide/Imine</td>
<td>2 1739.23 C = O Amide/Imine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 4</td>
<td>1522.68 C=C Aromatic</td>
<td>3 1621.36 C = C Aromatic</td>
<td>4 1398.63 C-H Alkane</td>
<td>4 1369.89 C – H Alkane</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1239.75 C-O ester</td>
<td>5 1220.73 C - O Ester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2 (Acetic acid 0.3M)</td>
<td></td>
<td></td>
<td>No Commercial gelatin (Merk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3275.90 O – H Carboxylic acid</td>
<td>1 3274.92 O - H Carboxylic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1626.97 C = O Amide/Imine</td>
<td>2 1628.26 C = O Amide/Imine</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>1522.74 C = C Aromatic</td>
<td>3 1524.12 C = C Aromatic</td>
<td>4 1438.4 C – H Alkane</td>
<td>4 1230.27 C – O ester</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1233.81 C - O Ester</td>
<td></td>
<td></td>
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</table>

Source: Result analysis using FTIR
Figure 3 illustrates FTIR spectra of each treatment is almost the same. Absorption curves show absorption widened 3268 cm\(^{-1}\) at T1, 3275 cm\(^{-1}\) in T2, 2969 cm\(^{-1}\) in T3 and 3274 cm\(^{-1}\) on commercial gelatin (Table 2). Wide peak shape was evidence of an OH group of hydroxyproline [21]. Further explained, most of the peaks N-H free-absorbed to have a narrow and sharply at 3650-3580 cm\(^{-1}\). When the NH group of a peptide binds hydrogen, then the position will be shifted to a lower wave number and NH bond overlap with the OH group of the region which cause broad absorption.

Subsequent absorption peaks for T1, T2, T3, and commercial gelatin is 1630 cm\(^{-1}\), 1626 cm\(^{-1}\), 1739 cm\(^{-1}\) and 1628 cm\(^{-1}\). The area demonstrates a strain of C = O and OH groups competing with carboxyl groups. The area absorption is known as the residual absorption imide. Subsequent uptake is peak aromatic (C = C) at wave number 1522 cm\(^{-1}\), 1621 cm\(^{-1}\) and 1524 cm\(^{-1}\) for commercial gelatin.

On the curve, following uptake presents absorption peaks in 1398 cm\(^{-1}\) for T1, T2 for 1438 cm\(^{-1}\), 1369 cm\(^{-1}\) in T3, for gelatin commercial not visible as it is covered by the OH peak.
Overall the spectral curve bovine split hide gelatin produced with different concentrations of acetic acid have the same absorption curve shape with commercial gelatin, but different in the absorption peak wave number.

**CONCLUSION**

Molecular weight distribution, and the amino acid profile of the functional group bovine split hide gelatin treated with different concentrations of acetic acid is almost the same as the commercial gelatin. The higher concentrations of acetic acid to 0.3 M produces gelatin better properties. Gelatin cowhide split with acid process could substitute commercial gelatin.

**ACKNOWLEDGMENTS**

The authors would like to thank Ministry of Industry on the financing support through educational programs S3 degree (doctorate).

**REFERENCES**


