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Evaluation of gelatin characterization of Bali cattle hide based on the FTIR approach and molecular weight

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Abstract
The aim of this research is to develop gelatin from protein extraction from bali cattle hide and to evaluate its conformation through the FTIR approach and the molecular weight of gelatin. The research method used a completely randomized design with a factorial pattern (4 x 3), namely acetic acid (A1=2%; A2=4%; A3=6% and A4=8%) and curing time (C1=2 days; C2= 4 days and C3 = 6 days). Each treatment was repeated 3 times. The results showed that the results of the FTIR test, gelatin from the extraction of collagen protein from the hide of bali cattle tested positive as a protein and was identified as a hydroxyl, carbonyl and amide group. The FTIR test results are in the frequency range (cm-1) between 500 – 3500. Meanwhile, the molecular weight study using the SDS-PAGE method is predicted to range from 67-135 kDa. The conclusion of the study is that the effect of using different concentrations of acetic acid and different curing times has not given a different spectrum of changes to collagen protein in bali cattle hide. Meanwhile, a high concentration of acetic acid and a longer curing time causes shorter protein degradation by producing a lower molecular weight.

Keywords: Bali cattle hide, gelatin, FTIR, molecular weight

Introduction
Gelatin is a product of the hydrolysis of collagen protein in animal skins and bones. The benefits of gelatin are very broad both for food and non-food. So far, this type of leather from Bali cattle has not been studied optimally for its potential as a raw material for gelatin. Meanwhile, Bali cattle are one of Indonesia's Nuftah plasmas and their distribution is very wide in Indonesia as livestock that are adaptive to the environment. The potential for Bali cattle hide to be processed into gelatin is very large. However, one of the obstacles to the extraction of bali cattle skin is the increase in skin swelling prior to hydrolysis to gelatin. Puspawati *et al*. (2012) [8] stated that the extraction of collagen protein from the skin of broiler chicken feet by starting with the curing process with acetic acid at a concentration of 1.5% for 3 days produces gelatin with optimal quality through analysis indicators FTIR (Fourier Transform Infra-Red).

Curing Broiler Chicken Leg Skin Curing Cattle Leg hide Curing Goat Leg Hide

Fig 1: Condition of cattle leg skin after curing acetic acid (1.5%) for 3 Days (Miwada dan Simpen, 2014) [3]

However, this method has not been able to maximize the extraction of collagen protein from the calf skin (Miwada and Simpen, 2014) [3].

The results of the preliminary research in Figure 1. can be observed and proven that a concentration of 1.5% acetic acid with curing for 3 days has not been able to cause calf leg skin and swollen goat leg skin, while broiler chicken leg skin looks to have maximum swelling. If the development process is not optimal, then this condition is thought to have an impact on the low quantity and quality of collagen protein extracted to become gelatin (Miwada *et al*., 2015) [4]. Determining the optimal extraction method for the skin of Bali cattle is very important so that optimal gelatin is produced.

Material and Methods

Experimental design: The main material of the research was bali cattle hide obtained from the Bali Badung Mammal Slaughterhouse, acetic acid, ethanol, the plasticizer used was glycerol (Brataco chemika). The characterization of gelatin from bali cattle hide consisted of 2 treatment factors, namely the concentration of acetic acid (A) including A1 (2%); A2 (4%); A3 (6%); and A4 (8%) while the second factor is the long curing time (C) including C1 (2 days); C2 (4 days) and C3 (6 days). Each treatment used 3 repetitions.

Gelatin Production

Research on the manufacture of gelatin from the skin of Bali cattle using a modified method according to Miwada *et al*. (2017) [5]. Bali cattle skin after being skinned, that is, taken from the side of the krupon or the back and washed until completely clean, drained, and cured as much as 1 kg each according to the treatment using acetic acid solution with a concentration of 2%; 4%; 6% and 8% and curing time 2 days; 4 days and 6 days, then washed until completely clean (until it shows a neutral pH or a negative test for the pp indicator). The acid-cured balinese cow skin was extracted with ethanol (1:1) for 1 hour to minimize fat. After extraction was carried out for 1 hour, followed by washing, filtering, evaporation of the extracting solution, and coagulation of the obtained gelatin product and drying. Gelatin samples ready for FTIR and molecular weight testing.

Observed variables

Gelatin active function group test (Hashim *et al*., 2010) [2]

FTIR analysis is used to determine the specific functional groups of prepared gelatin. The gelatin sample used is gelatin powder obtained through a variation of the soaking process in 2% acetic acid solution; 4%; 6% and 8% (w/v) with curing times (2 days, 4 days and 6 days). As much as 2 mg of sample was mixed into 100 mg of potassium bromide (KBr) powder and homogenized then KBr discs were made. Then the samples were scanned in an FTIR device in the range of 4000-500 cm-1 with a resolution of 4 cm-1. The resulting IR absorption was compared with commercial gelatin.

Gelatin Molecular Weight Test with the SDS-PAGE Method

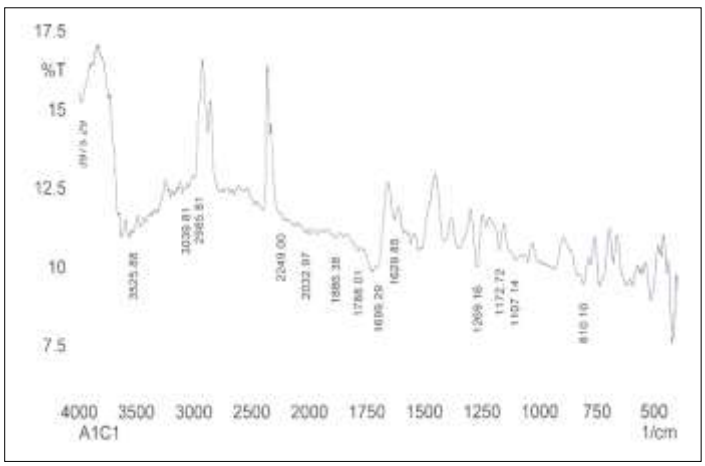
A total of 50 mg of sample was dissolved in 1.0 mL of buffer solution (250 mM Tris-ClpH7.5; 5 mM EDTA; 2% SDS), then heated at 85oC for 1 hour. After that, the solution was mixed with 0.5 M tris-HCl sample buffer, pH 6.8 (containing 4% (w/v) SDS, 20% (v/v) glycerol, and 10% (v/v) βME) at a ratio of 1: 1 (v/v). Then the mixture was heated to 100 ºC for 3 minutes. Samples were put into a polyacrylamide gel made with 7.5% (v/v) running gel and 4% (v/v) stacking gel. Electrophoresis was carried out at a constant current of 15 mA, then the gel was stained with buffer staining 0.1% (w/v) Coomassie blue R-250 in 15% (v/v) methanol and 5% (v/v) acetic acid and destained with 30% (v/v) methanol and 10% (v/v) acetic acid.

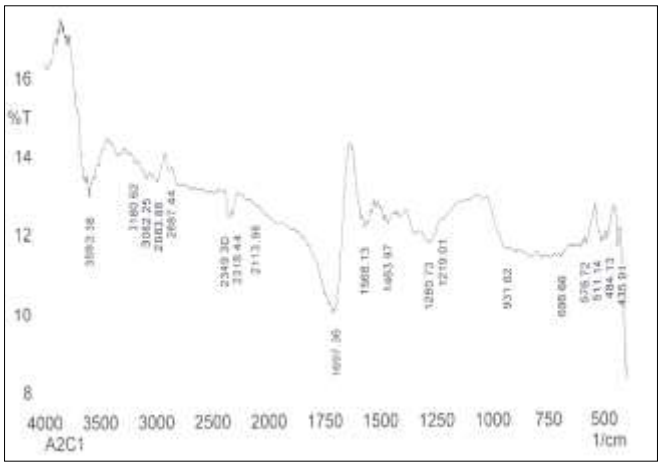
Statistical analysis

The data from this study were analyzed for variance with a completely randomized factorial design, and continued with a descriptive analysis of the FTIR variable and the resulting gelatin molecular weight.

Results

Extraction of collagen protein in cattle hide is done by treating balinese cattle hide swelling (Swelling). This process is carried out by soaking (Curing) the skin of a bali cattle with acetic acid. To ensure that what is extracted is gelatin, it is proven by conducting FTIR testing. The results of the study are presented in full in Figure 2.





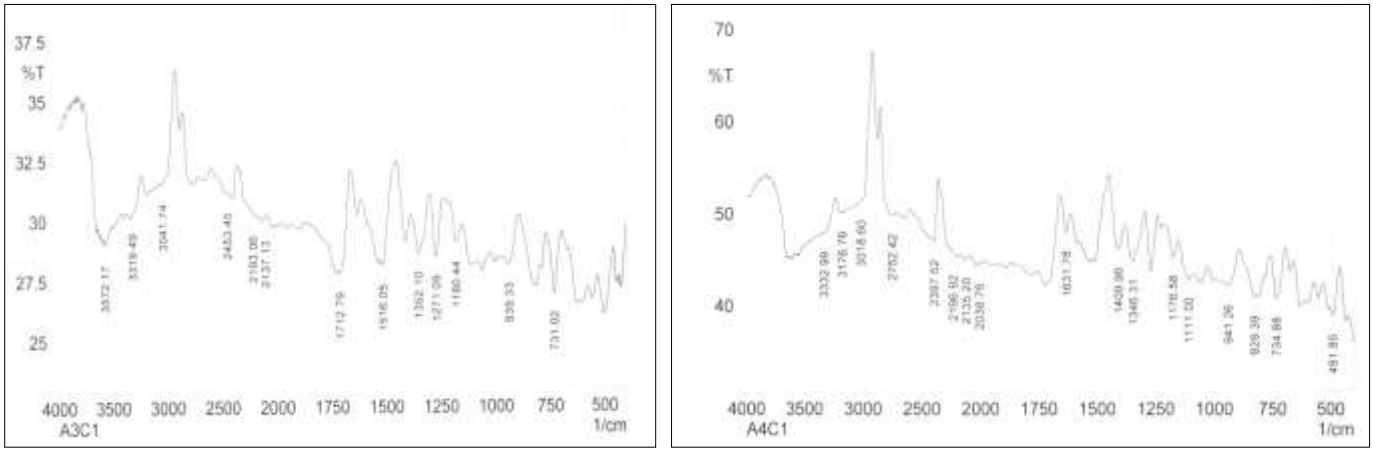


Fig 2: FTIR Test of Bali Cattle Hide Gelatin Different Acetic Acid Concentrations and 2 Days Curing

Based on the test results with the FTIR approach (Figure 2) that the characteristics of gelatin from bali cattle hide with the same curing time of 2 days (C1) and different concentrations of acetic acid (2%=A1C1; 4%=A2C1; 6%=A3C1 and 8%=A4C1) indicates that the quality of the gelatin meets the category as the extracted protein part. Likewise, in Figure 3.

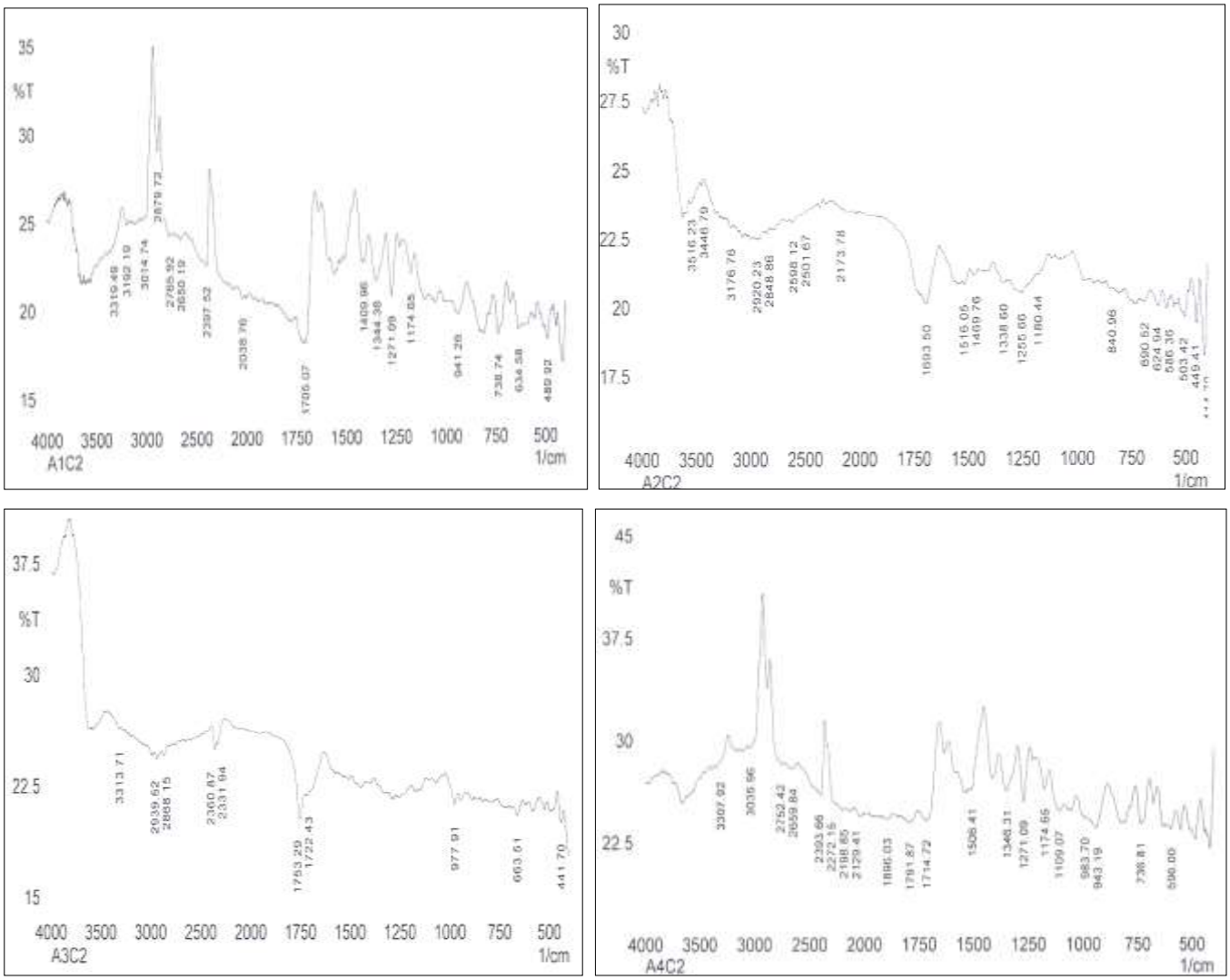


Fig 3: FTIR test of bali cattle hide gelatin with different concentrations of acetic acid and curing for 4 Days

Characteristics of gelatin from collagen protein extraction from bali cattle skin during 4 days curing with different concentrations of acetic acid (2%=A1C2; 4%=A2C2; 6%=A3C2 and 8%=A4C2), as shown in Figure 3, does not different from curing 2 days (Figure 2). The FTIR test indicator shows that the quality of the gelatin meets the category of the extracted protein part. Testing at the following stages is also the same as in Figure 4.

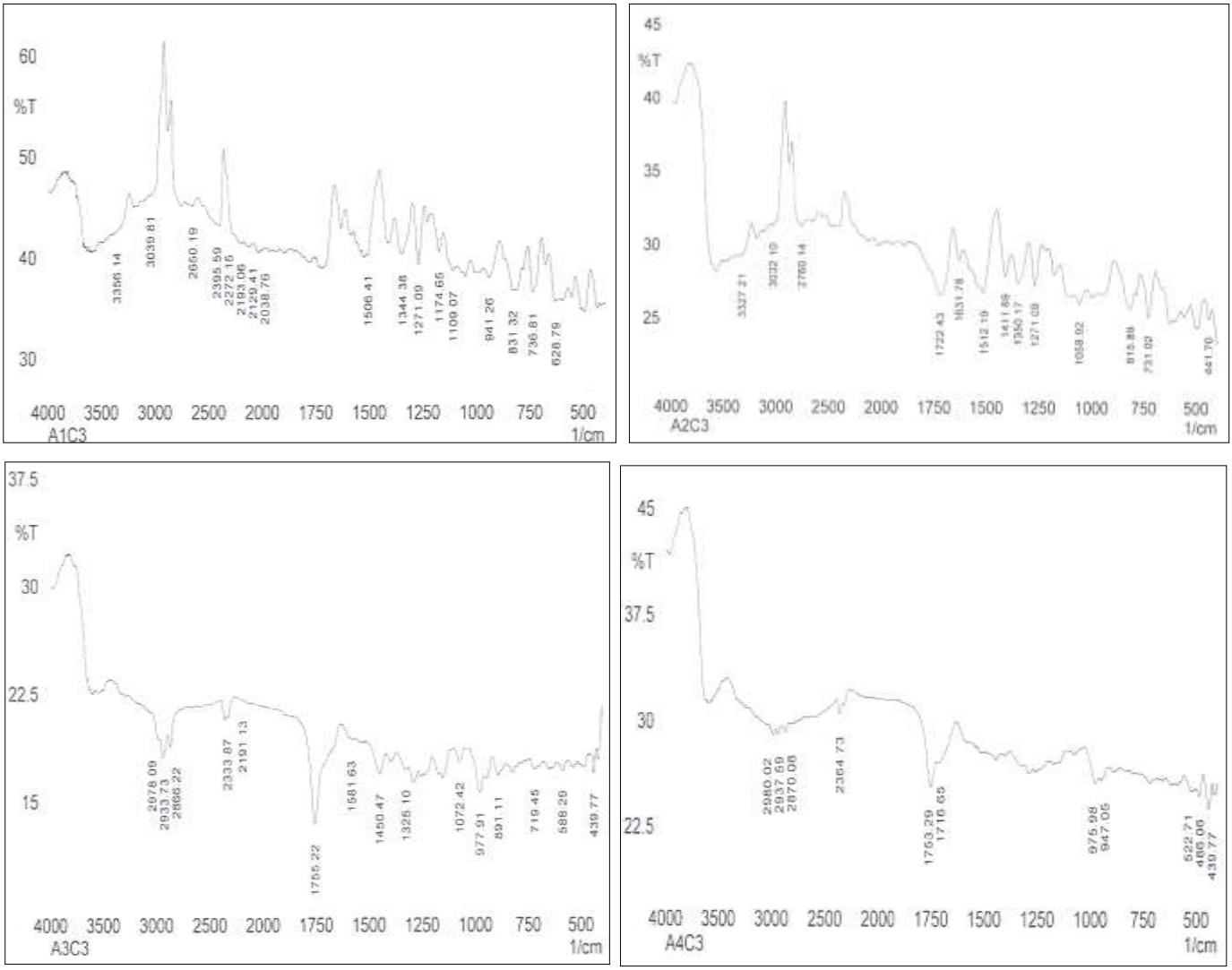


Fig 4: FTIR test of bali cattle hide gelatin with different concentrations of acetic acid and curing for 6 Days

Discussion

Proof of gelatin extracted from the skin of bali cattle curing 6 days with different concentrations of acetic acid (2%=A1C3; 4%=A2C3; 6%=A3C3 and 8%=A4C3) shows the same thing, especially in the functional components making up gelatin. Observations in Figures 2 – 4 show that the spectrum of gelatin is very clear in each treatment with the same characteristics. The test for estimating the gelatin content above was carried out using the FTIR approach. (Fourier Transform Infrared). The test results in Figure 2 – 4 show that the frequency area (cm-1) is in the range between 500 – 3500. In this range, functional groups were found in gelatin from bali cattle hide extract, namely the hydroxyl, carbonyl, amide types and all of these are categorized as components of protein (gelatin).

The effect of different treatment concentrations using acetic acid and different curing times has not given a different spectrum of changes to the collagen protein in cattle hide, especially in the process of making gelatin. The results of observations of the typical gelatin absorption peak curve in Figure 5.1-5.3 show that 4 amide regions were found, namely amide A, amide I, amide II, amide III. Absorption area of amide A at ν = 3600-2300 cm-1, amide I at ν = 1636-1661 cm-1, amide II at ν = 1560-1335 cm-1, and amide III at ν = 1240-670 cm-1 (Muyongga, 2004) [6]. It is stated that the amide A region indicates the presence of NH groups and indicates the presence of hydrogen bonds. The amide B region indicates the presence of a CH group. The amide I region shows the presence of a C=O group which is the secondary structure of the protein. While the amide II region shows the presence of NH bonds and the amide III region shows the presence of N-H bonds which shows the presence of a helical structure.

Based on these results it can be seen that the infrared spectrum identifies the presence of collagen protein structures in gelatin. The secondary structure of collagen is a three-dimensional protein structure that describes the relationships between atoms affected by non-covalent bonds such as hydrogen bonds. This is also corroborated by the opinion of Puspawati *et al*. (2012) [8] who stated that gelatin, like proteins in general, has a structure composed of carbon, hydrogen, hydroxyl groups, carbonyl groups and amine groups. Therefore, the extraction of collagen protein from the skin of Bali cattle by applying the treatment in this research proved that the resulting gelatin is a structure of the protein itself.

The molecular weight of gelatin resulting from the extraction of collagen protein from the skin of bali cattle was carried out using the SDS-PAGE method approach. The SDS-PAGE measurement results of Bali cattle skin gelatin treated with acetic acid concentration and curing time are presented descriptively in Figures 5 and 6. In Figures 5 and 6, it can be seen that the bands or bands of gelatin protein molecules in each treatment are in almost the same position with the range of bands from thin to very thick. The effect of different acetic

acid concentrations and curing times has a different effect on the decomposition of collagen protein molecules in the hide of bali cattle. The higher the concentration of acetic acid and the curing time causes the chain

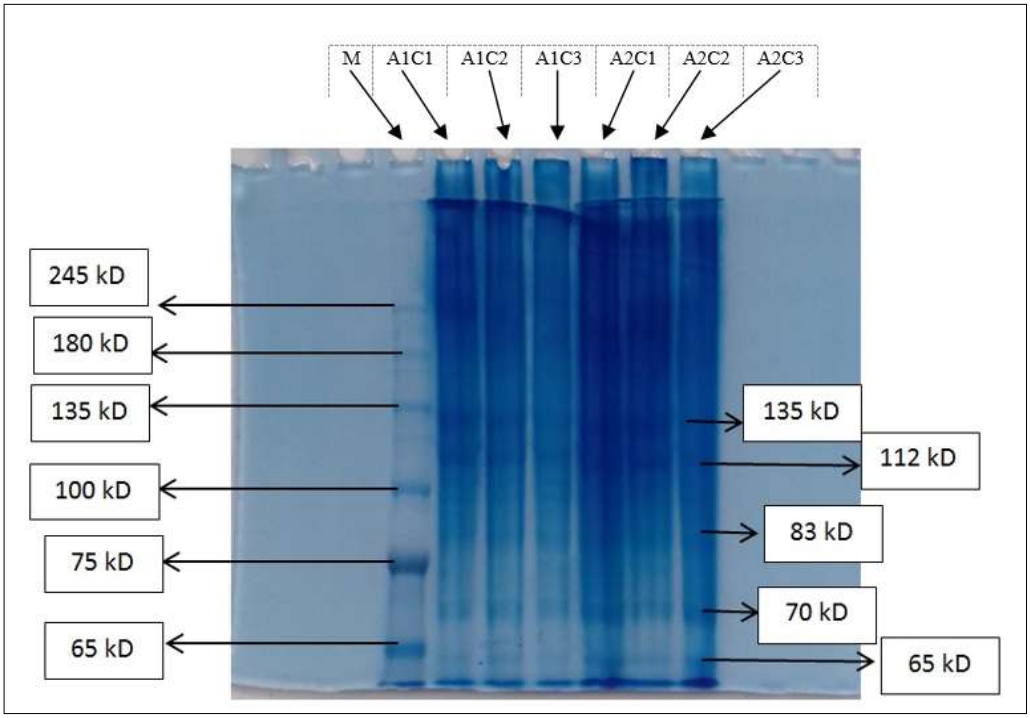


Fig 5: SDS-PAGE test results for bali cow skin gelatin at different treatments with acetic acid concentrations (2% and 4%) and curing time

shorter polypeptide breaks so that the molecular weight is low. Sarbon *et al*. (2013) [9] stated that the molecular weight of gelatin protein is affected by hydrolysis factors which play a role in peptide breaking and intramolecular cross-linking between peptide chains. The thickness of the protein band or band indicates the concentration of the protein where the protein with a thicker intensity has a higher concentration. Fig 5. shows the lowest band thickness level in the range of 70 kDa while in Figure 6, the lowest band thickness level is read at 67 kDa. Therefore, a high concentration of acetic acid and a longer curing time causes shorter protein degradation by producing a lower molecular weight and a lower molecular weight is an indicator that maximum protein degradation has occurred

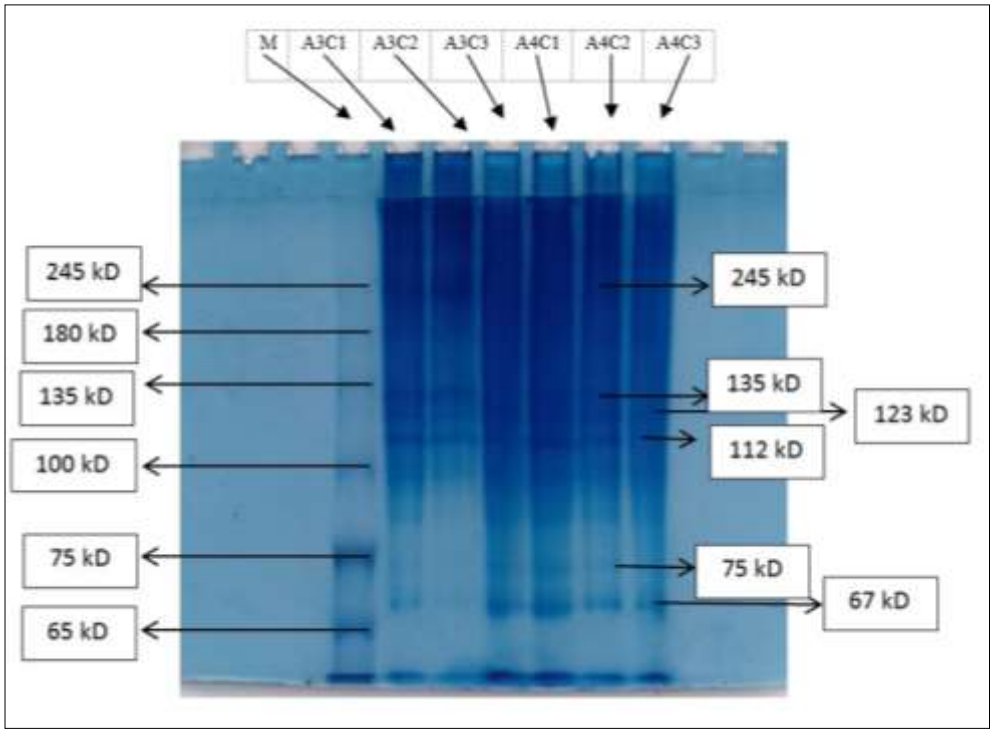


Fig 6: SDS-PAGE test results for bali cow skin gelatin at different treatments with acetic acid concentrations (6% and 8%) and curing time

Molecular weight is related to the properties and ability to form gelatin, namely the higher the molecular weight, the better the resulting gelatin (Sobral and Habitante, 2001) [10]. Molecular weights detected by SDS-PAGE showed clear protein bands between treatments with a calculated range of molecular weights ranging from 67-135 kDa. These results prove that the purity level of the gelatin protein is still under control after swelling or swelling of the skin of bali cattle by curing treatment with acetic acid concentrations and different times.

The results of the study with FTIR (Figures 2-4) and the molecular weight test of gelatin showed that the conformation of the gelatin protein did not differ in the range of using acetic acid or using the curing time as in the treatment above, proving that the gelatin obtained in the process of extracting the skin of this bali cattle still quite good. Denavi *et al*. (2009) [1] and Mulyani *et al*. (2017) [7] stated that protein conformation and protein level determine the hydrophobic, ionic, hydrogen bonding, and the interactions that can be built between protein chains.

Conclusion

The results of the study of the estimation of gelatin from the extraction of collagen protein from the skin of Bali cattle using the FTIR approach. (Fourier Transform Infra-Red) shows that in the frequency range (cm-1) between 500 – 3500, namely the presence of hydroxyl, carbonyl, amide groups and all of these are categorized as components of protein (gelatin). Meanwhile, the study of the molecular weight of gelatin from the extraction of collagen protein from the skin of bali cattle using the SDS-PAGE method showed the lowest band thickness was in the range of 67-70 kDa. The high concentration of acetic acid and the longer curing time causes the protein to be degraded shorter by producing a lower molecular weight.

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Conflict of interest declaration

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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