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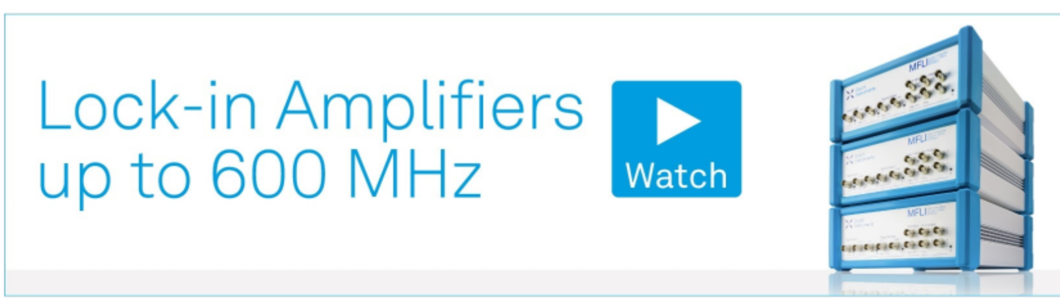

Characterization of Gelatin from Scapula (Os scapula) from Aceh Cattle

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Abstract

High protein content in bones of animals, especially collagen, made them good sources for gelatin extraction. The purpose of this study is to determine the physicochemical properties of gelatin compounds using the scapula (Os Scapula) of cows. The extraction process has been done by using the scapula of the cattle bone, which was obtained from cattle slaughter in Langsa, Aceh. The extraction was done by acid hydrolysis method using 10% of acetic acid with 9 days demineralization time. The obtained gelatin was analysed using FTIR. The physico-chemical analysis showed that the obtained gelatin has yellow colour with 4.39% of yield, 1.08% of water content, 4.67% of ash content, 92.88% of protein, 7.31 cP of viscosity, and pH 5.6. Based on the FTIR spectrum of gelatin showed identic signals with the gelatin standard, such as O-H stretching and O-H bending around 3393.60 and 1435.04 cm^{-1}, C-H stretching and bending at 2930.73 cm^{-1}, C-H aromatis at 3078.39 cm^{-1}, C = O stretching at 1655.89 cm^{-1}, respectively and the N-H stretching has similar value with O-H signal.

Keyword : Gelatin, Scapula *(Os Scapula)*, Aceh Cattle

INTRODUCTION

Meugang is a tradition of the Aceh people for welcoming the holy day in Islam such as welcoming Eid al-fitr, Ramadhan fasting, Eid al-Adha and Mawlid an-Nabi. At the time of meugang, the beef requirements became more demanding than usual day. In 2013, the cattle requirement is about 5487 heads with an average of 15 heads / day while at Meugang for welcoming fasting the cattle requirements can reach 61 cattle per day [1]. Seeing the number of cows that are cut per day, then many bovine bones are produced. If this wasted material was not utilized, it can disrupt the environment by the several unwanted impacts [2,3]. But if the wasted material can be utilized, it can increase the economic value of the shoulder blade (*Os scapula*) of cattle to be used as a raw material of gelatin [4].

Gelatin is obtained by the hydrolysis processing of collagen, the main tissue building of vertebrate animals. The high protein content of the cattle bones, especially collagen, opens a lot of opportunity to produce gelatin [5]. Collagen is obtained from mammals from bones and animal skins. Based on its sources, gelatin is produced from pig

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raw material is about 41%, cow leather 28.6%, bone 30% and others 0.4%, with the total world production reached 269,000 tons [6]. Based on data obtained that the import of gelatin in Indonesia is increasing every year, in 2004 is around 2,639,692 kg and in 2008 to 3,764,856 kg [7].

Gelatin extraction and characterization have been widely performed by researchers from bone and skin of mammals, poultry, and fish by acidic and basic methods [8–14]. Gelatin that obtained from the mammals gives the best quality compared to other sources such as fish, poultry and marine [15-16]. Gelatin obtained from mammalian bones meet the national standards of Indonesia and can be used in the pharmaceutical industry [17]. Gelatin has transparent, colorless / yellowish, brittle (dry), easily damaged, powder-shaped, tasteless solids, soluble in hot water and acetic acid, also gelatin is difficult to dissolve in organic solvents . Based on the literature review, the conducted research was about determination the physical and chemical properties of scapula Oscella gelatin oct cattle using acetic acid (CH_3COOH) 9% method, in the further the obtained gelatin will be used as an encapsulation material.

EXPERIMENTAL

Materials

Beef bones (Os Scapula) were obtained from the Slaughterhouses (RPH) Langsa Aceh and acetic acid glacial was obtained from Merck. The equipment used in this research, such as desiccator, grinding machine (Mod M-2300B), hot plate, waterbath, oven blower (DHG - 9076A Shanghai), pH meter, FTIR Spectrophotometer (Perkin Elmer 1600 series FTIR), and Ostwald Viscometer.

Gelatin extraction from cow bone

Gelatin extraction was performed by acid hydrolysis method according to [11,17] with some modifications. Extraction was performed using 1500 grams of cow bone that has been cleaned from the remains contaminants. The bones were boiled at 60-70 $^{\circ}$C for 3 hours to remove the fat and then soaked in acetic acid 9% (1: 1.5 b / v) for 8 days. The treated bone was washed with water until the neutral pH was obtained. The obtained results were extracted with hot water at 60-70 $^{\circ}$C, 70-80 $^{\circ}$C and 80-90 $^{\circ}$C for 3 hours. The boiled water of the bone was filtered, and the obtained filtrate was thickened in a waterbath. The obtained gel was dried in an oven at 60 $^{\circ}$C until gelatin was formed and blended with a blender into powder was obtained. Furthermore, physical and chemical properties, functional groups (FTIR) were tested.

Characterization of Physical Properties and Chemistry of Gelatin

The yield of Gelatin

The yield was obtained from the weight ratio of dry gelatin produced with the total scapula (Os scapula) of fresh cows (bones that have been cleansed of meat and fat residue). The yield can be obtained by using calculation according [18]

$$\text{Yield (\%)} = \frac{\text{mass of dried gelatin}}{\text{mass of fresh bones}} \times 100\% \qquad (1)$$

pH

One percent of the gelatin powder was dispersed in 100 ml of aquadest at t 80 ºC. Gelatin is homogenized with a magnetic stirer. Then the degree of acidity (pH) is measured at room temperature with pH meters.

Water content

Gelatin powder is then weighed 1 gram and placed in a cup that has been weighed. The cup + sample was placed in an oven at 105^{0}C for 2 hours, then it was cooled in a desiccator until the weight is constant. Water content is obtained by using the following equation:

$$W\% = \frac{M_1 - M_2}{M_1} x100\% \qquad (2)$$

Information:
W = Percentage of water content
M1 = sample weight before being dried
M2 = sample weight after drying

Ash content

One gram of gelatin was put into a crucible which has been weighed then put in furnace with a temperature of 600 ºC and cooled in a desiccator. Ash content is calculated by formula

$$\text{ash content } (\%) = \frac{\text{mass of ash}}{\text{mass of sample}} x100\% \quad (3)$$

Viscosity

Gelatin solution with concentration of 6.67% (w / w) was prepared with aquadest, then the viscosity of that solution was measured using the Brookfield Syncro-Lectric Viscometer tool. Measurements were made at 60 ºC at a rate of 60 rpm.

Relative molecular mass

Gelatin powder was weighed about 0.03 grams and dissolved in 10 ml of aqueous solvents (aquadest) at room temperature. The solution was then measured using the Ostwald viscometer. The flow time of solution and solvent was measured using stopwatch five times.

Protein

Protein content was determined by micro-kjeldahl method. A total of 0.2 g of gelatin was weighed and put into a 30 ml kjeldahl flask. Then added 2 gr of K_2SO4, 50 mg HgO and 2.5 ml of H_2SO_4 into the flask. The samples were destructed for 1-1.5 hours until the clear green liquid was obtained, then the solution was cooled, and the distilled water was added slowly. The contents of the flask are transferred into the distillation apparatus, 10 ml of concentrated NaOH was added into the apparatus until the color turned to blackish brown, then it was distilled. The distillation product was kept in a 125 ml Erlenmeyer containing 5 ml of H_3BO_3 and titrated with 0.02N HCl until a pink change occurred. The protein content was calculated using formula below (12):

$$N(\%) = \frac{(\text{mL HCl} - \text{mL Blank})x14{,}007xN\ \text{HCl}}{\text{mg Sample}} x100\% \quad (4)$$

$$\text{Protein } (\%) = N(\%)x6{,}25 \quad (5)$$

RESULT AND DISCUSSION

The yield of gelatin that obtained from extraction process is about 4,39%. The yield value of this research is between the value from research conducted by Hajrawati [14] which ranged between 2.27-4.87%. The low yield of gelatin is caused by the incomplete hydrolysis of collagen [19] and the rate of conversion of collagen proteins is affected by the several factors such as temperature, time and pH [20]. If the value of yield is high, that indicated the production process is done more efficiently [21]. The obtained of cow bone gelatin can be shown in Figure 1 below.

FIGURE 1. Gelatin Extraction Result

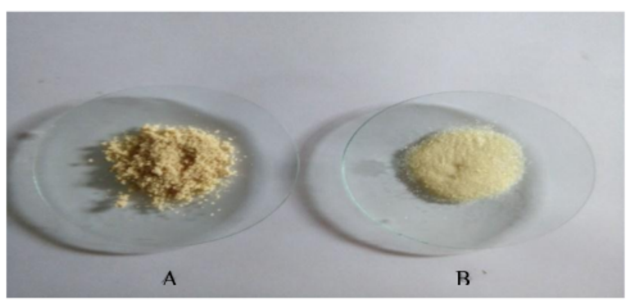

FIGURE.2 Color Gelatin

The results of the physical and chemical analysis of the obtained gelatin can be shown in Table 1 as follows:

TABLE 1. Physical and Chemical Properties of Bone Gelatin of the Cow of Aceh

No	Sample	Gelatin Isolates	Commercial Gelatin	GMIA 2012
1	Water Content (%)	1.08	0.90	8-13
2	Ash content (%)	4.67	1.17	0-3.2
3	Protein (%)	92.88	88.59	85-92
4	Viscosity (cP)	7.31	17.84	1,5-7
5	pH	5.60	5.10	4.5-6.5

Water content

Water content in the food is very important to determine because it can influence the texture and ability of the material against damage caused by microbes. From Table 1, the resulting gelatin water content is about 1.08% higher than the commercial gelatin water content of 0.90% so that the value still meets the quality standard specified which is maximum 16% and 8-13% [22]. The low water content of gelatin is caused by the time of the drying process is long enough, about 72 hours with the temperature 50^0C, that treatment cause the water content in gelatin runs out. In addition, it is also influenced by the high concentration of acid that used in during the treatment. That acid can increase the production of H^+ ions and it can hydrolyze the collagen. Consequently, the protein peptide bond and the resulting polar group are able to bind the water contained in the gelatin. Water content in gelatin will affect the quality of gelatin. The quality of gelatin depends on the physicochemical properties, which is influenced by species, tissues and methods of processing [9].

Ash Content

Ash content is very important determined in a foodstuff which aims to see the level of purity and hygiene of a product from inorganic substances so that the quality of the resulting material is guaranteed. From Table 1, the gelatin content of cows' shoulder blade was 4.67% higher than commercial gel ash content of 1.17 and gelatin ash content based on GMIA[22] ranging from 0-3.2%. The high levels of cow bone gelatin ash indicate the demineralization process was not optimal. This indicates that the resulting gelatin still contains many minerals such as Ca, Mg and Fe that has not been released during the washing. This is in accordance with Astawan and Aviana [23] studies that the high ash content is due to the presence of non-collagen components at the time of gelatin extraction. High levels of ash tend to give a darker color of the product. The lower ash content of a product will be better because it affects the final color of the product. The color of gelatin from the cow's shoulder tends to be more yellow (2A) compared to commercial gelatin color (2B). This can be shown in Figure 2 above this paragraph.

Protein

According to GMIA [22], protein content of gelatin is 85-92%. From Table 1, the gelatin content of the cow's bones is 90.26% higher than the commercial gelatin protein content of 88.59% and the protein content of Said, et al (3) is 73,9%. This is because the raw materials have high protein content. According to Said (3), the scapula (os scapula) of the cow has a very high protein content of 1/3 of the scapula composition composition or approximately 60-70% of collagen-containing protein. Therefore, in the process of immersion with acid, the collagen will be easily hydrolyzed. The reaction that occurred with the acid will help colagen to open the coil structure and the amount of extracted protein becomes high. High acid concentration causes an increase in the concentration of H + ions in solution which will eventually accelerate the hydrolysis process.

Viscosity and Molecular Weight

Viscosity has the ability to withstand from a liquid to flow due to the adsorption and development of a colloid and may affect other gelatin properties such as emulsion stability and gel formation. Gel properties in gelatin are strongly influenced by viscosity. The formation of gels that have high viscosity will be better than low gelatin viscosity. The value of the viscosity affects the molecular weight of the gelatin determined by the length of the amino acid chain contained in the gelatin. The viscosity and molecular weight of gelatin from the cow's bones was 7.31 cp and 22729.65 g / mol lower than the commercial gelatin viscosity of 17.84 cP with molecular weight of 56619.23 g / mol. Based on this research it appears that the relative viscosity is directly proportional to the molecular weight. Beef gelatin isolation results show relatively smaller viscosity and BM than commercial. It is allegedly because the high acid concentration causes the amino acid chain structure to be more open and bonding occurs due to decrease gelatin viscosity

pH value

Gelatin solution has different pH. Jamilah [19] states that the difference in pH of gelatin is influenced by the difference of acid type and strength used in gelatin making process. Using a pH meter, the pH value produced by gelatin isolates was 5.6, it is higher than the commercial gelatin pH of 5.1 but still in the range of standard [22]. This is thought to be due to the length of the immersion process carried out during the demineralization process. Giving acid in the immersion process will cause bone collagen fibers will swelling (swelling) so that the opening on the bond structure of amino acids in collagen and acid will be trapped between the bonds. In the process of neutralization, the acid molecule is not soluble especially in the washing process is less than optimal so there are still residual acid immersion results carried during the extraction that can affect the resulting pH value. It is also caused by the acid concentration used. The higher the acid solvent concentration used, the resulting gelatin acidity will increase. In accordance with the opinion of Astawan and Aviana [23], the pH value of gelatin is influenced by the type of acid used at the time of immersion. If the acid used is a strong acid type, the pH value of the resulting gelatin is low and vice versa.

Gelatin Functional Groups Analysis

In addition to its physical properties and chemical properties, gelatin can be determined by its functional groups which can be determined by FTIR analysis. This analysis aims to determine the functional groups of gelatins. In general, the gelatin structure contains functional groups contained in proteins i.e. hydroxyl (O-H), carbonyl (C = O), and amine (N-H) groups. The results of FTIR analysis of gelatin of cow and commercial gelatin bones in this study are shown in Figure 3 below:

Based on the infrared spectra of isolated gelatin from Fig.3, it shows the presence of v (O-H) stretching at 3393.60 cm^{-1} wave numbers. The wave number 1435.04 cm-1 indicates the presence of v (O-H) bending. The presence of OH groups is from water that used in the gelatin extraction process. Stretching and bending v (C-H) is represented by the wave number 2930.73 cm^{-1}. The aromatic C-H peak at wave number 3078.39 cm^{-1}. Stretching v (C = O) is present at the wave number 1655.89 cm^{-1}. Peak v (N-H) stretching is not visible because it is covered by OH peak. The infrared spectra of commercial gelatin in Fig. 3 shows vibration of stretching v (O-H) at wave number 3394.60 cm^{-1}. Bending v (O-H) is indicated by the wave number 1450.47 cm^{-1}. Stretching and bending v (C-H) are represented by wave numbers 2943.66 cm^{-1}. The aromatic C-H peak at wave number 3086.11cm^{-1}. Peak v (C = O) stretching with wave number 1648.13 cm^{-1}. Peak v (N-H) stretching is not visible because it is covered by OH peak. The results of the infrared spectra show that the wave numbers of the isolate and commercial gelatin groups have almost no difference at all. This proves that the compounds produced in this study are gelatin. This is consistent with Martingingsih [13] that from the FTIR analysis of gelatin from Pari fish skin showed that the wave number v (O-H) stretching at 3441.2 cm^{-1}. Bending v (O-H) is indicated by the wave number 1448.10 cm^{-1}. Stretching and bending v (C-H) is indicated by the wave number 2928.50 cm^{-1} and the aromatic C-H peak is shown at the 3048 cm^{-1} wave number and the peak v (C = O) stretching is indicated by the 1650 cm^{-1} wave number. The peak of NH stretching is not visible because it is covered by the OH peak. Thus, it can be concluded that the spectra found in commercial fish and cow gelatin contain functional groups of O-H, C-H, C = O, N-H and C-H aromatic and similar to functional groups of cow rotate gelatin.

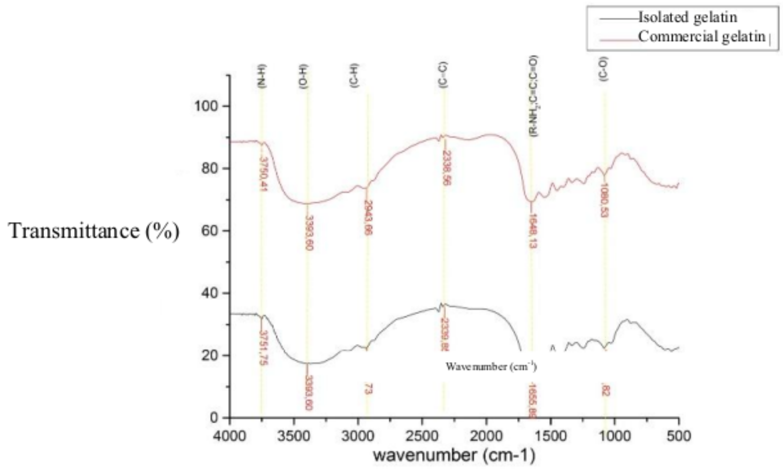

FIGURE 3. FTIR analysis of gelatin of cow and commercial gelatin bones

CONCLUSIONS

Cows's bones from Aceh can be extracted into halal gelatin with the functional groups of O-H, C-H, C = O, N-H and C-H aromatic. Based on its chemical properties, the obtained powder of gelatin is yellow with characteristic, 4.39% of yield, 1.08% of moisture content, 4.67% of ash content, 92.88% of protein, 7.31cP of viscosity, and 5.6 of pH.

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