

Fourier Transform Infrared (FTIR) Spectra, Amino Acid Profile and Microstructure of Gelatin From Madura and Crossbred Ongole Cattle Hides

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ABSTRACT

The research was aimed to extract and determine the characteristics of gelatin from Madura and Crossbred Ongole cattle hides prepared using bases and acid curing. The research materials were rawhide from 2.5-3 years of age males Madura and crossbred ongole cattle, curing solutions were sodium hydroxide (NaOH 0.25 M) and hydrochloric acid (HCl 0.25 M). The data obtained were descriptively analyzed which includes a functional group using FTIR (Fourier Transform Infrared Spectrofotometri), amino acid profile using HPLC (High Performance Liquid Chromatography) and gelatin microstructure using SEM (Scanning Electron Microscopy). The commercial gelatin was used as a control standard gelatin. The results showed the intensity of the infrared absorption of main functional groups were O-H, C=O, C=C, C-H and C-O and there were different functional groups absorption intensity between Madura, Crosbreed Ongole cattle hides and commercial gelatin. Amino acid profile showed that histidine was the highest amino acid on all the gelatin samples from Madura-Base, Madura-Acid, Crossbred Ongole-Base, Crossbred Ongole-Acid and Commercial gelatin and the hystidine content were 32.16, 22.34, 29.79, 34.84 and 18.74 g/100g respectively. The lowest amino acid content prepared gelatin was methionine (0.01 g/100g) while the commercial gelatin was tyrosine (0.22 g/100g). The glycine amino acid was founded on the prepared gelatin but was not founded on commercial gelatin. Scanning Electron Micrograph showed on all samples had not different appearance. Inconclusion, gelatin from cattle hide (Madura and Crossbred Ongole) using base (NaOH 0.25M) and acid (HCl 0.25M) curing have different characteristics.

Keywords: Gelatin characteristics, Madura, Crosbred Ongole cattle hides, base and acid curing.

INTRODUCTION

Gelatin is a derivative protein from collagen fibers obtained in skin, chicken claws and connective tissue, where the raw material is rich in collagen as the main raw material for collagen production. There are two kinds of gelatin that is distinguished from the manufacture process, namely gelatin type A (acid) and type B (base). The acid process is carried out by soaking the raw material by curing or acidic solutions such as HCl, formic acid, acetic acid and others while type B with basic solutions such as NaOH or lime solution (Ca (OH₂)). Both acid and base solutions are used in gelatin making, each of which has deficiencies and advantages, including the acid process requires a relatively faster time and higher yield than the base process, but the quality of gelatin is higher when using base processes. In addition to the raw

materials used, the process of making gelatin will affect the quality of gelatin product (Schrieber and Gareis, 2007).

The raw material from hide, commonly faster prepared than from the bone. Some studies have shown that gelatin from pig skin has better quality than cow hide, but in the majority Muslim community, the use of pig skin as a raw material of gelatin is prohibited. Therefore it is necessary to do a lot of halal gelatin research with halal raw materials, one of which is cow hide. This research has been conducted by previous researchers include gelatin goat skin with curing CH_3COOH and $\text{Ca}(\text{OH})_2$ (Said, 2011), the skin of the legs of broilers with age cut differences and temperature extraction differences (Taufik (2011) and much more research on gelatin. Indonesia has the potential of local cattle, such as Madura cattle and Crossbreed Ongole cattle with special characteristics but the potential of local cattle hides has not been developed as a raw material of gelatin. There were no detail data about the characteristics, profile, and quality of gelatin from hides of Indonesian local cattle. Along with the increasing needs of gelatin in various fields such as food, cosmetics, health and so forth, it is necessary to study and research on the hides of Indonesian local cattle as a raw material gelatin, using either the curing acid and curing the base to determine the characteristics and The quality of gelatin to be produced, both with Physicochemical test and see the profile of amino acids (with HPLC) and gelatin microstructure, such as with FTIR (Fourier Transform Infrared) or SEM (Scanning Electron Microscope).

MATERIALS AND METHODS

The research materials were rawhide of Madura cattle and male Crossbreed Ongole cattle about 2.5 to 3 years old, croupon (shoulder) and body (back). Curing solutions were sodium hydroxide (NaOH 0.25 M) and hydrochloric acid (HCl 0.25 M and commercial gelatin) Functional group profile analysis by FTIR (Fourier Transform Infra Red) according to Sastrohamidjojo (1992) and Sastrohamidjojo (2001) brand / type: ABB MIRacle MB 3000, amino acid analysis with HPLC Waters Alliance system 2695 and SEM TM3000 Hitachi analysis with Quorum Q150RS coating and Swift ED3000. The method of making gelatin is Madura cattle hides (M) and Crossbreed Ongole Cattle hides (P) soaked in acid curing (A) and base (B) in the ratio (1: 2) for 2 h, washed to normal pH (7), extracted with aquadest in waterbath (1: 2) with temperature 55°C for 6 h, in oven temperature 60°C for 4 days (until dry) and Mashed with a blender The data in the form of graph (FTIR), composition table (amino acid) and image (SEM) were analyzed descriptively based on the tested samples.

RESULTS AND DISCUSSION

FTIR

FTIR was tested for the identification of a chemical compound or confirmation of a chemical compound through its functional group or functional group profile present in gelatin, wherein gelatin has a typical functional group exhibiting a certain chemical bond containing gelatin. Rohman (2014) explained that FTIR spectrophotometers were capable of providing spectra of compounds in the range of $4000\text{-}400\text{ cm}^{-1}$ waves because they were the most important areas for qualitative analysis of organic systems, in which most vibrations or basic vibrations are found in the area The range of such wave numbers. The results of the analysis of the research samples were commercial gelatin (K), from Madura cattle hides with acid curing (PMA), base (PMB), from crossbreed Ongole cattle hide with acid curing (PPA) and base (PPB) can be seen in Figure 1

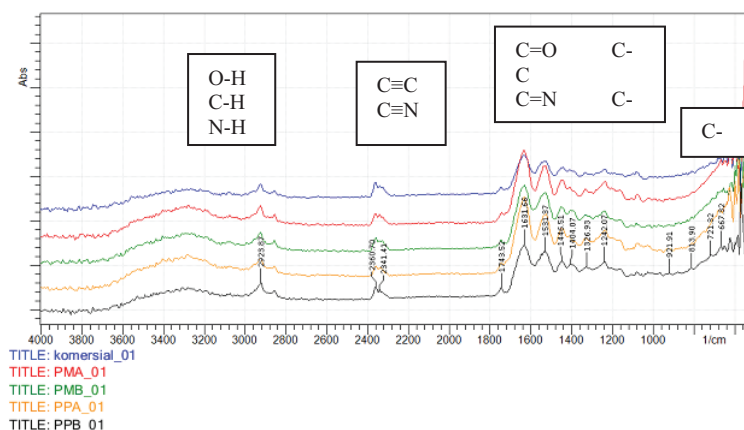


Figure 1. Graph of intensity absorption of gelatin functional groups of 5 research samples

The figure showed that the pattern of infrared absorption intensity of the five research samples of gelatin had an identical shape but the intensity was different, it was because the ability of each sample is different in absorbing the intensity of infrared beam (Sastrohamidjojo, 1992). The result of infrared absorbance of the sample shows that infrared spectrum has detected the presence of a number of main functional groups, OH, C-H, C = O and C-O, both in commercial gelatin, gelatin from Madura cattle hides and crossbred cattle hides using acid and base curing. The OH, CH and NH functional groups absorb infrared at wave numbers 4000-2500 cm^{-1} (ie 2923 cm^{-1}), the functional groups C = C and C \equiv N absorb infrared at the wavelength 2500-2000 cm^{-1} (ie 2360 and 2341 cm^{-1}), the functional group C = O absorbs infrared at 1800-1650 cm^{-1} wave numbers (ie, 1743 and 1631 cm^{-1}), the functional groups C = N, C = C, and N = O absorb Infrared at the wave number 1650-1550 cm^{-1} (ie 1591, 1446, 1404, 1326 and 1242 cm^{-1}) and the functional group CX absorbs infrared at wave numbers less than 1000 cm^{-1} (ie 921, 815, 721 and 667 cm^{-1}). Compounds having the same functional groups tend to have the same chemical reaction. The presence of functional groups is related to the structure of amino acid molecules that make up gelatin, which is structurally composed of a number of amino acids. These amino acids contain functional groups. Functional groups detected other than derived from their constituent structures (amino acids) are also likely derived from the curing material used (Anonymous, 2009).

Muyonga et al. (2004) explained that the peak curve of gelatin absorption was divided into 4 parts, ie the of the amide A at ν (3600-2300 cm^{-1}), the amide I at ν (1636-1661 cm^{-1}), the absorption area of the amide II at ν (1560-1335 cm^{-1}), the absorption area of the amide III at ν (1300-1200 cm^{-1}). From the FTIR spectra analysis results, it can be seen that the gene groups contained in the research sample were OH, C-O, N-H groups of secondary amides supported by the presence of C -N, C = O and NCO groups of secondary amides as main gelatin functional groups.

Amino acid

Amino acids are organic compounds having carboxyl functional groups (-COOH) and amines (-NH₂). Gelatin is a collagen derived that generally has a collagen-like amino acid composition (Gimenez et al., 2005; Nemati et al., 2003). The fifth amino acid profile of the research sample can be seen in Table 1.

Table 1. Amino acid composition of Madura cattle hides, Crossbreed Ongole cattle hides, and commercial

No	Amino acid (g/100 g)	Madura		Crossbreed Ongole		Commercial
		Acid	Base	Acid	Base	
1	Asp	2.33	2.57	3.34	3.33	2.01
2	Ser	0.82	1.16	1.19	1.09	0.64
3	Glu	4.35	5.81	5.79	5.47	3.82
4	Gly	0.12	0.47	0.52	0.45	*
5	His	22.34	32.16	34.84	29.78	18.74
6	Arg	4.67	5.21	5.04	4.42	4.30
7	Thr	0.78	0.89	0.55	0.51	0.38
8	Ala	5.53	5.81	6.57	5.64	4.72
9	Pro	5.91	8.52	9.29	7.95	6.40
10	Cys	1.09	0.42	0.46	0.43	0.58
11	Tyr	0.21	0.51	0.45	0.51	0.22
12	Val	1.00	1.53	1.52	1.44	1.24
13	Met	0.01	0.01	0.01	0.01	0.93
14	Lys	1.44	2.28	2.11	2.03	1.63
15	Ile	0.66	1.14	1.05	1.05	0.79
16	Leu	1.48	1.61	1.57	1.55	1.44
17	Phe	1.00	1.96	2.10	1.99	0.95
18	Hyp(%)**	0.37	0.40	0.35	0.29	0.33

Description: * = not detected. ** = spectrophotometer

All of the gelatin samples showed that histidine was the largest amino acid compared to other amino acids. The smallest histidine content was on a commercial gelatin sample of 18.74 (g / 100g) and the largest was from gelatin from Crossbreed Ongole cattle hides with acid curing of 34.84 (g / 100g). Amino glycine is not detected in commercial gelatin but in other samples detected and ranges from 0.12-0.45 (g / 100g). The smallest amino acids from Madura cattle gelatin and Crossbreed Ongole cattle gelatin, both with acid curing as well as bases are methionine ie only 0.01 (g / 100g).

SEM

Gelatin characterization also can be seen by looking at gelatin gel microstructure by using SEM. Research samples as a Figure 2.

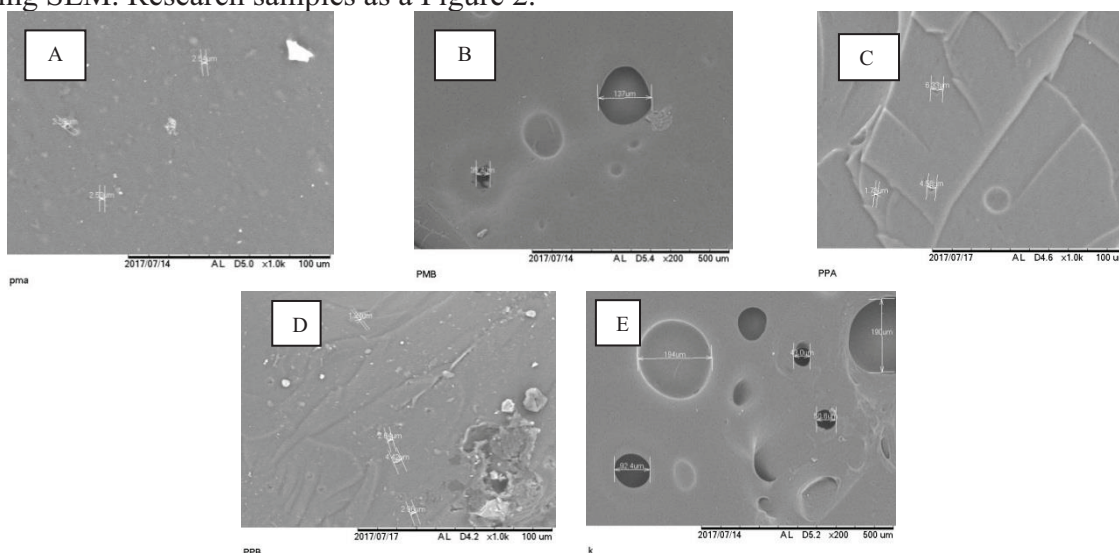


Figure 2. SEM results in five research samples with 200x magnification

A: PMA (200x magnification); B: PMB (200x magnification); C: PPA (1000x magnification); D: PPB (1000x magnification); E: Commercial (K) (200x magnification).

Figure 2 showed the gelatin microstructure of the research compared with commercial gelatin indicating that gelatin from both Madura cattle hides and Crossbreed Ongole Cattle hides with acid curing as well as base is able to form a closer microstructure compared to commercial gelatin. This can be seen from the larger size of the cavity in commercial gelatin. Gelatin from Madura cattle hides with acid curing has a larger cavity compared to gelatin from Crossbreed Ongole Cattle hides. Fadillah et al. (2014) stated that microstructure (SEM test) gelatin gel from chicken scratch with 5% HCl curing for 30 minutes has the potential to be a natural preservative of meat and fish because it can form a thin layer of dense so that it can be used as a coating for food preservative.

CONCLUSIONS

Gelatin made from Madura cattle hides and Crossbreed Ongole Cattle hides with acid curing (HCl 0.25 M) and base (NaOH (0.25 M) for two hours produce an infrared absorption intensity pattern with identical shapes but varying intensities. The largest Amino acids are histidine and the smallest is methionine and produces different gelatin gel microstructures.

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