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Research Article

Characteristics and Antioxidant of Goat Skin Gelatin Pretreatment with Lactobacillus plantarum 1UHCC and Acetic Acid

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ABSTRACT

Goatskin with lactobacillus plantarum 1UHCC pretreatment, and acetic acid are expected to be able to explore goat skin gelatin at different ages so that it has high quality and health benefits as an antioxidant. The quality of the resulting goat skin gelatin was evaluated of its antioxidant content using the Diphenylpicrylhydrazyl (DPPH) method, protein content, fat content, water content, Fourier Transform Infrared Spectroscopy (FTIR). A combination of different goat ages with L. plantarum pretreatment results in a higher antioxidant effect. Protein and fat levels and other chemical properties of gelatin influence antioxidant activity, as well as changes in functional groups of gelatin using Fourier Transform Infrared Spectroscopy (FTIR), detected four amide areas was amide group A (3566.38 - 2854.65 cm-1), amide group I (2358.94 - 2126.56 cm-1), amide group II (1452, 40 cm-1) and amide III group 1080.14 - 669.30 cm-1. The existence of hydrogen bonds allows the formation of free radical bonds in gelatin. In conclusion that L. plantarum I uhcc pretreatment at 1-year-old goat skin has the highest antioxidant content. Likewise, the chemical properties of goat skin gelatin were strongly related to the antioxidant activity of gelatin.

Keywords: Goat, skin, Gelatin, antioxidant

INTRODUCTION

Gelatin is a polypeptide extracted from collagen which is found in connective tissue such as skin and bone[1].. Gelatin plays a major role to increase the nutritional value and quality of a product, [2]beneficial to health as an antioxidant, antihypertensive and anticancer. Gelatin as an antioxidant has been widely studied, one of which [3]has examined the antioxidant effect of pig collagen. But Muslims do not consume any ingredients from pigs because they are not halal so it is needed to find other sources of gelatin that are halal and safe for Muslims.

Goat skin is rich in protein compounds such as collagen, tightly bound with calcium mineral which has the potential to be processed into gelatin [1]According to goat skin contains a gelatin range of 11.50%-16.39% goat bones between 11.99-14.69% [4]. Goat skin has a thick, hard fur texture and a distinctive odor so that it is underutilized. Processing goat skin waste into gelatin can increase added value and reduce waste pollution.

The physical and chemical properties of gelatin are influenced by the age of the animal, type of animal, method of manufacture, characteristics, type of collagen, treatment processes such as temperature, pH and time [5].Making gelatin through an acid immersion process causes intramolecular and intermolecular bonding structures in the skin collagen protein to weaken so that partially breaking of the amino acid bonding chain occurs[6].Pretreatment with chemical acids using acetic acid (CH_3COOH) and biological acids using L. plantarum 1UHCC are expected to function partially. as curing material. L. plantarum in the fermentation process can be used in increasing the production of lactic acid [7].The purpose of this study was to determine the initial potential of antioxidant of goat skin gelatin, determine the effect of acid pretreatment on gelatin extraction and to determine the optimal age of goat skin.

Materials and Methods

Goat skin waste of etawa crossbreed, aged 1, 2 and 3 years, was obtained from the Wessabbe goat business in Maros Regency, South Sulawesi, Indonesia. Goat skin coated with lime paste to facilitate the extraction process for 30 minutes then cleaned of feathers, the remaining meat and fat using a knife. Furthermore, the goat skin was cut into small size 1x1 cm, washed and then dried using a 50°C oven for 24 hours, packaged and stored at room temperature 27°C until the extraction process. All chemicals used are proanalysis (PA).

MATERIALS

Gelatin Extraction Raw Material Screening Phase

The raw material of goat skin (200g) was fermented using L. Plantarum 1UHCC culture of 5% concentration into MRSbroth media (1: 3w / v). Positive control used a CH_3COOH solution of 5% concentration (1: 3 w / v), the use of various sources of acid as the main plot and various ages of the goat as the subplot. Gelatin production using an acid source follows the method (Ockerman and Hansen 2012) with modification. Samples of various ages were mixed into the L.plantarum 1UHCC culture media then put into an erlenmeyer (1:3 w/v) and covered with gauze, the mixing process was carried out in laminar air flow. Samples were then fermented using a shaker for 72 hours at a room temperature of 28°C. The immersion time was given from the best results of previous studies (24, 48 and 72 hours) and the selected immersion time is using L. plantarum 1UHCC for 72 hours while the immersion time using CH_3COOH for 24 hours. The sample was then washed with water (1:5 w/v) three times then filtered and then squeezed and put into an erlenmeyer, plus distilled water (1:4 w/v) following the method [8]with modification.

Gelatin Extraction Optimization Stage

Raw material (200g) from L.plantarum 1UHCC fermentation concentration of 5% (main plot X1, N) for 72 hours and the results of immersion of the sample using CH_3COOH concentration of 5% (main plot X1, N) respectively from the age of goats 1, 2 and 3 years (subplot X3, Years). Each sample was washed using water (1:5 w/v) three times, filtered then squeezed then the sample was put into a beaker and added with distilled water (1: 4 w / v) then covered using aluminum foil following the method (Da'i,2010) with modification. Furthermore the sample was extracted using a water bath at 70°C for 24 hours then the extraction results were filtered and poured in a baking dish and then dried using an oven at 70°C for 48 hours to form solids.

METHODS

Antioxidant levels using the DPPH method

Samples concentration series of 1 mL and control were added 1 mL DPPH each, the volume is sufficient 5ml with methanol, and incubated in 30 minutes in a dark room, then measured with a maximum wavelength (515 nm) using spectronic 20D + [12]

The DPPH method was chosen because it has several advantages namely accurate, practical, fast, simple and easy for screening free radical scavenger activities (Fahleny and Wini, 2014). IC_{50} (Inhibition Concentration) is a parameter used to interpret DPPH test results. IC_{50} is a concentration of sample solution that causes a reduction in DPPH activity by 50% [9].

Protein levels

0.5 g sample was added with ¼ bussino tablet, 12 ml H_2SO_4 and then crushed in a FOSS ± 410°C tube for 1 hour. Crushed samples were distilled with 4% H_3BO_4, thio-NaOH 40%, BCGMR Indicator. A total of 150 ml was distilled in an Erlenmeyer disk and titrated with 0.099 N HCl until the color changed from blue to pink. The value of 5.55 is used as a conversion factor for gelatin protein. Protein content formula (%) = ((ml HCl - ml Blank) x N HCl x 14.008 x 100 x 5.55) / g sample x 1000 [10].

Fat levels

A sample of 2 g was added with ether then put into Soxhletfl and dried at 105°C for 2 hours. Soak in water for ± 3 hours to use the reflux process then dried in the oven for 1 hour at 105°C, and cooled in a desiccator. Fat content formula (%) = (fat weight (g) / sample weight (g)) x100% [4].

Fourier Transform Infrared Spectroscopy (FTIR) spectra

Gelatin powder as much as 2 mg is made into pieces in 100 mg of potassium bromide (KBr) and then pressed at a pressure of 8-20 tons / unit area to obtain pellet shape. KBr in dry condition are crushed under an infrared lamp to prevent condensation of vapors from the atmosphere and then vacuum to release water. The pellet is then put into the sample chamber on an infrared spectrophotometer and the spectrum results will be read through a computer monitor. The analysis process uses a Fourier Transform Infrared Spectrophotometry(FTIR) (Shimadzu PC-8201) wave range distance of 4000 to 650 cm-1[11].

Data Analysis

The split plot design in a randomized complete design with SPSS statistics 21 program was used to see the effect of gelatin extraction on L. Plantarum 1UHCC pretreatment and acetic acid (CH_3COOH) with various ages of goat skin. If the Anova analysis results show the effect then proceed with the Duncan test.

Gelatin antioxidant levels qualitatively can be known from the decay reaction in samples that have been added by Diphenylpicrylhydrazyl (DPPH), as stated by [12]that samples containing antioxidants if Diphenylpicrylhydrazyl (DPPH) is added will decrease the color intensity of the solution (purple) according to the inhibition and concentration of ingredients that contain antioxidants.

RESULTS AND DISCUSSION

Antioxidant levels using the DPPH method

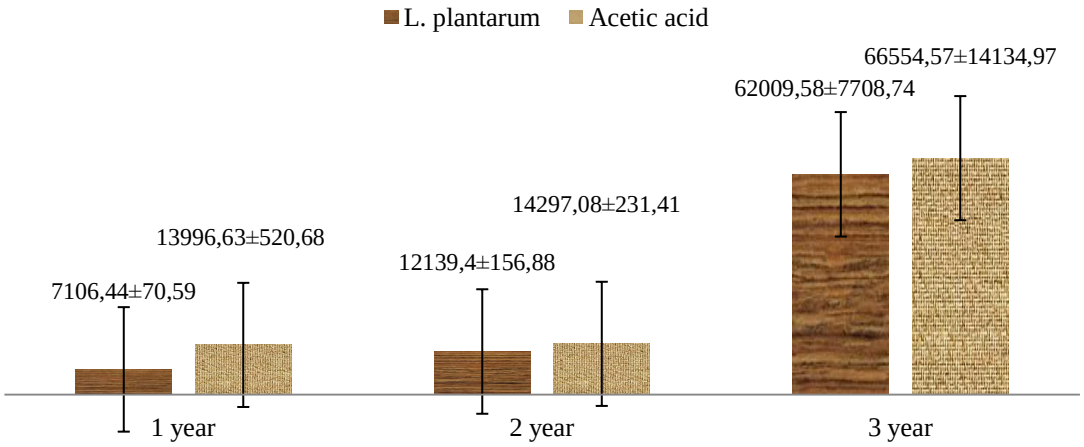
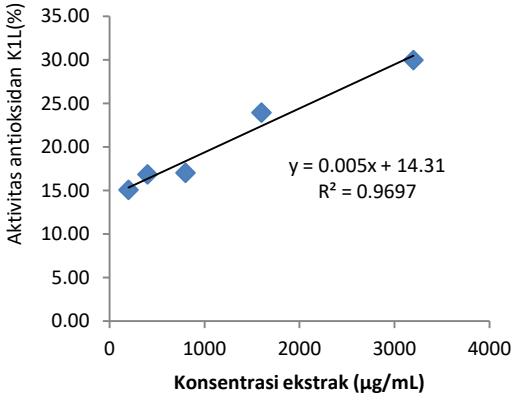
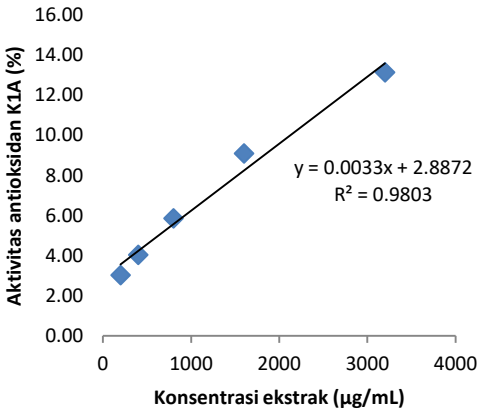


Fig.1:IC_{50} value of antioxidant (%) goat skin gelatin L. plantarum 1UHCC and acetic acid (CH_3COOH) in goat skin age (1, 2 and 3 years)





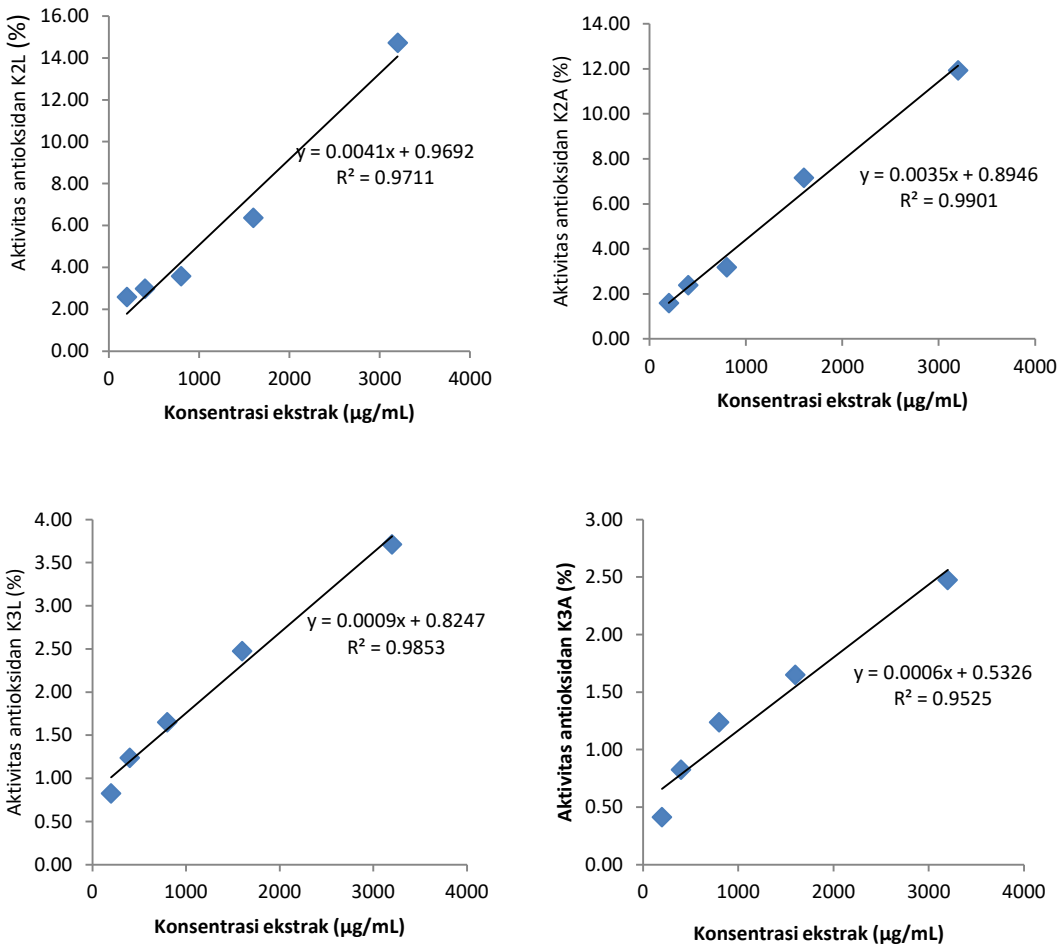


Fig.2. Antioxidant absorption value (%) of goat skin gelatin. Y. Linear Regresssion Equation

The results of measurements of the antioxidant activity of goat skin gelatin in fig 1. Shows that L. plantarum 1UHCC treatment is higher than acetic acid (CH_3COOH) treatment although it is not significant ($P>0.05$), but the age of goat (1, 2 and 3 years) shows a very significant effect ($P<0.01$) respectively IC_{50}10551.53±295.64µg/mL; IC_{50} 13218.24±194.15µg/mL;IC_{50}64282.08±10921 .85 µg / mL. A low IC_{50} value of 1 year old goat skin gelatin means it has high antioxidant compared to 2 and 3 years old, showing greater potential for capturing free radicals[13].

Fig 1. shows initial identification of antioxidant content in goat skin gelatin. The capture of free radicals by antioxidant gelatin is formed from hydrogen bonds [14].The presence of hydroxy (-OH) group (Fig. 4) in gelatin protein is bound to aromatic amino acids and generally in peptides contain aromatic amino acids such as glycine, proline and hydroxyproline which play an important role in antioxidant activity([15].

Protein Levels

Gelatin protein level is obtained from how big the content of collagen which is hydrolyzed into gelatin[16].

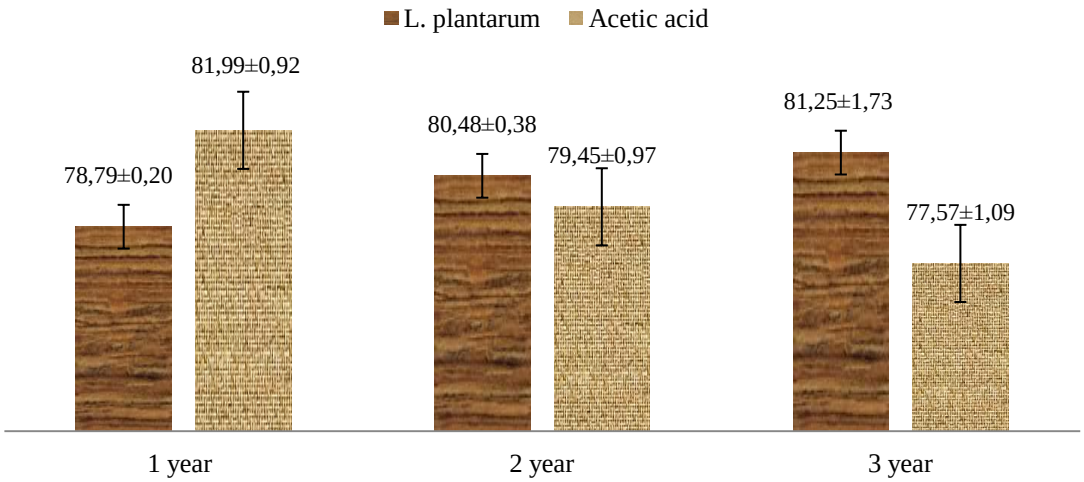


Fig.2:Protein levels of goat skin gelatin pretreatment L. plantarum 1UHCC and acetic acid (CH_3COOH) at different ages

The results of measurement of goat skin gelatin protein levels are presented in Fig. 2. Shows the existence of the same interaction or response between acid treatment (L.plantarum 1UHCC, acetic acid (CH_3COOH)) 80.17±0.77%; 79.67±0.10% with age of goat skin successively 80.39±0.56%; 79.96±0.68%; 79.41±1.41%. The acid treatment used shows the dissolution of collagen protein[10].Protein content of gelatin is directly related to the chemical properties of gelatin such as antioxidants contained, changes in secondary structure and functional groups of gelatin (Sultanaal., 2018). Increasing the amount of dissolved protein causes the level of gelatin protein to increase and more amino acid bonds make up the broken protein[1].

Fat Levels

Based on fig 3, it shows L. plantarum 1UHCC treatment of goat skin gelatin fat content is lower 0.25±0.03% compared to acetic acid (CH_3COOH) 0.48±0.02%. The higher age of goats, the fat content of gelatin is also higher, respectively 0.16±0.02%; 0.44 ±0.02%; 0.50±0.04%.

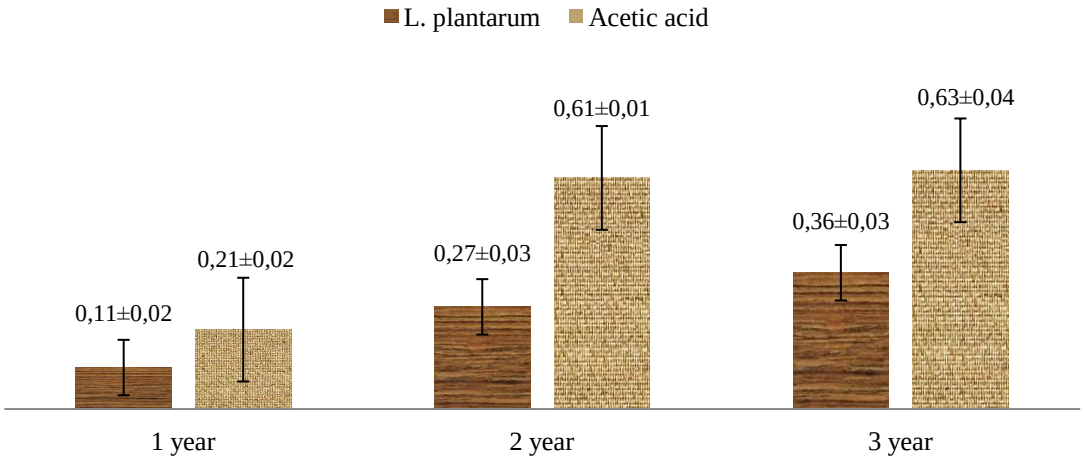


Fig.3:Fat Levels of goat skin gelatin Pretreatment of L. plantarum 1UHCC and acetic acid (CH_3COOH) at different ages

Increasing levels of gelatin fat along with the increase in goat age, showed a higher fat content of 3-year-old goats compared to ages 1 and 2 years. Increasing age of livestock causes the formation of subcutaneous fat increases[18].Raw materials used in the process of making gelatin affect the levels of gelatin fat (Said al., 2011b). Gelatin raw material that contains high fat content tends to produce high levels of gelatin fat as well [5].

Acetic acid (CH_3COOH) pretreatment showed a higher fat level rather thanL.plantarum1UHCC treatment. [1]caused by the treatment of acetic acid (CH_3COOH) the more fat binding protein molecules that dissolve and are deposited on the collagen proteins that make up gelatin so that the levels of gelatin fat increase. The process of soaking with acid will cause fat to separate from connective tissue [16].A different statement was stated by [19].that the value of fat content is quite high due to the process of degreasing that has not been maximized. It is supported by the statement of [40]the high level of gelatin fat is thought to be caused in the degreasing process at 70 °C has not been able to separate the fat content in the bone optimally, so that the resulting gelatin still contains quite high fat. High temperatures will result in decreased fat density so that fat will float on the surface [16].

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra are used to identify secondary structures and functional groups of gelatin [11]. It can also be used to provide further information about the chemical composition and the corresponding peptide fraction (Gomes al., 2010). FTIR goat skin gelatin starts from 4000 cm^{-1} to 500 cm^{-1} (fig 4).

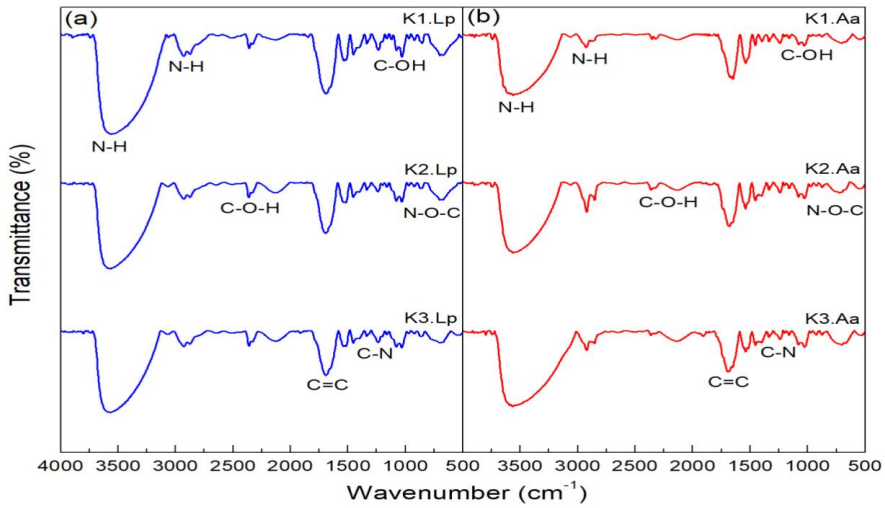


Fig 4. FTIR spectra gelatin of goat skin with pretreatment using L. plantarum 1UHCC and Acetic Acid (CH_3COOH).

1 year goat skin gelatin L. plantarum 1UHCC pretreatment was not much different from acetic acid pretreatment, detected at wave number 3562.52 cm^{-1} whereas 2 and 3 year goat skin gelatin showed no difference but L. plantarum 1UHCC pretreatment (3566, 38 cm^{-1}) shows the difference with acetic acid (3564.45 cm^{-1}).

Amide A goat skin gelatin in the numbers 3566.38 - 2854.65 cm^{-1}, this result is not much different from the research Muyongaal., (2004) who reported that the identification of the amide A region in the wave number 3600-2300 cm^{-1} . The stretch of N-H bonds at the amide A peak indicates the presence of hydrogen bonds (Mulyanial., 2017). Amide A value with wave number 3562.52 - 2875.86 cm-1 at the age of 1 year with L.plantarum 1UHCC pretreatment (fig 4) approaching the vibration wave obtained[20].who reported wave numbers 3400-3440 cm^{-1} shows molecular degradation of gelatin. Gelatin polypeptides consist of two terminal atoms, a carboxyl group on the right end and an amino group on the left end. Both ends allow gelatin to form hydrogen bonds [16].Formation of hydrogen bonds in gelatin allows the capture of free radicals so that they are antioxidants[11].

Amide I gelatin of goat skin contained carboxylic acid hydrogen bonds with C-O-H bonds in fig 4 showing wave numbers at the age of 1 year L.plantarum 1UHCC 2358.94 - 2126.56 cm^{-1}. [17].argue that the amide I absorption is used to analyze the secondary structure of proteins. Amide I also represents a stretch of the C = C bond wave (1695.43 - 1519.91 cm^{-1}) at the age of 2 years of L. Plantarum pretreatment. The obtained wave length meets the gelatin coil structure according to the statement of [17].that the characteristics of the gelatin coil absorption wave crest structure are around 1633 cm^{-1}.

Amida II gelatin of goat skin with a peel of C-N bonding was obtained due to N-H vibrations[21].Age 1, 2 and 3 years in L.planratum 1uhcc pretreatment has the same

wave value of 1452, 40 cm^{-1} and acetic acid pretreatment 1456.24 cm^{-1} (fig 4).

Amide III fig 4 detected waves of 1080.14 - 669.30 cm^{-1} on goat skin gelatin by stretching C-OH (1080-1028.06 cm^{-1}) and NOC stretching on L. plantarum 1UHCC tended to be lower (698.23-673.16 cm-1) compared to acetic acid (704.02-669.30 cm^{-1}). Changes occur due to loss of the triple helix structure from denaturation results of collagen to gelatin[5].

CONCLUSIONS

Pretreatrment L. plantarum 1 UHCC on the age of 1 year goat skin has the highest antioxidant content. Likewise, the chemical properties of goat skin gelatin are strongly related to the antioxidant activity of gelatin.

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Competing Interests

The authors declare no competing interests.

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