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The Physicochemical Properties of Goat Skin Gelatin at Different Ages With the Use of *Lactobacillus plantarum* 1UHCC and Acetic Acid as a Pretreatment

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Abstract

Lactobacillus plantarum 1UHCC as a bacterium producing lactic acid and acetic acid (CH_3COOH) during the fermentation process on goat skin is expected to be able to breakdown proteins into simpler compounds so as to optimize the collagen denaturation process during gelatin extraction. Research purposes to determine the characteristics of goat skin gelatin at different ages of *L. plantarum* 1UHCC pretreatment and acetic acid (CH_3COOH). Each treatment was repeated 3 times. The study design used a Split Plot Design with acid source treatment as the main plot and the age of goats 1, 2 and 3 years as subplots. The results showed a very significant effect ($P<0.01$) between the age of the goat with gel strength, pH, viscosity and color. The highest gel strength and viscosity at 1 year of age with *L. plantarum* pretreatment respectively (441.37±0.78 g bloom) and (9.22±0.09 cP). Goat skin gelatin has the highest pH at 1 year of age with *L. plantarum* 5.24±0.16. Similarly, the effect of gelatin ($P<0.05$) with a brightness level (L) 76.38±1.10 and redness (a) 11.13±1.58 affect the age of goats and acidic sources while yellowish (b) 33.81±2.34 only affects the acid source. Age of goats with different acid pretreatment has an impact on the physicochemical properties of goat skin gelatin. One year old goat with *L. plantarum* treatment showed optimal results.

Keywords : Goat Skin, Gelatin, Age, *L. plantarum*.

INTRODUCTION

Goat skin has the most important constituent component called protein. Skin protein consists of collagen, elastin, globulin, keratin, mucin and albumin. For gelatin production, this collagen protein is utilized (Ockerman & Hansen, 2012)because gelatin is a protein denatured from collagen (Sompie *et al.*, 2012)

Several methods have been developed to obtain gelatin with better quality, and the most popular in pretreatment is using acids, bases and enzymes. Said *et al*. (2011)suggested the use of acid as a curing material because it produces a much better gelatin.

Pretreatment of chemical acids using acetic acid (CH_3COOH) has been widely carried out by many researchers (Mulyani *et al*., 2017; Said *et al*., 2011; Sompie *et al*., 2012) for use as a positive control. While biological acid pretreatment using microbial *L. plantarum* to form ossein in goat skin is still rarely done, while the application of Lactic Acid Bacteria (BAL) species of *L. plantarum* produce lactic acid which is able to breakdown protein compounds to be simpler. As the statement of Puspadewi*et al.*(2011)*L. plantarum* is a Gram-positive heterofermentative microaerophilic microorganism that is catalase negative and is known as a producer of high lactic acid.

Pretreatment of acid sources in goat skin is expected that *L. plantarum* and acetic acid bacteria during the fermentation process can break down proteins into simpler compounds so as to optimize the collagen denaturation process during gelatin extraction. This study aims to determine the characteristics of goat skin gelatin with age variations from *L. plantarum* 1UHCC pretreatment and acetic acid (CH_3COOH)

MATERIALS AND METHODS

Goat skin gelatin extraction

Goat skin is obtained from Wessabbe goat business in Taroada Village, sub-district of Turikale, Maros district with criteria male goats aged 1, 2 and 3 years. The skin is cleaned using tap water, then smeared with lime paste as thin as possible, and stored for 30 minutes, then the hairs and fat are completely cleaned. Then wash it with tap water until pH 7. (Riadh *et al.*, 2013)clean goat skin is cut into small pieces 1x1 cm in size. Furthermore, it is dried using an oven at 50^{o}C for 24 hours.

Gelatin production from pretreatment goat skin extraction uses acidic method (Ockerman & Hansen, 2012)with modification. Goat skin 1, 2 and 3 years that have been sterile washed using alcohol technical 70% then rinsed with distilled water. Each raw material was immersed in the culture plant *L. plantarum* 1UHCC concentration of 5% from the fermented milkfish and a solution of acetic acid 5%, for 72 hours. After being immersed the material is then neutralized using tap water to pH 7, weighed and extracted using a water bath at 70^{o}C for 24 hours then dried at 50^{o}C for 48 hours to form a solid gelatin sheet.

Gel Strength

6.67g of gelatin was dissolved in 100 ml of distilled water (w/v) then stirred using a magnetic stirrer, heated at 50^{o}C until the homogeneous solution was then stored in a refrigerator at 5^{o}C for 16-18 hours. Furthermore, it was measured using a TA-XT plus HD texture with a depth of 4 mm and a probe speed of 0.5 mm (Kaewudom, 2012).

Acidity(pH)

Gelatin samples were weighed 1g then dispersed into 10 ml distilled water which was heated at 50^{o}C and homogenized using a magnetic stirrer. The acidity of the solution was measured using an English standard pH meter 871 (Yang *et a.l.* 2018).

Viscosity

Gelatin powder was dissolved in distilled water at 50^{o}C with a concentration of 6.67% solution. Then stir using a magnetic stirrer until the solution is homogeneous, let stand until it reaches room temperature. The sample was measured using brookfield viscometer DV-1 prime (Arnesen & Gildberg, 2002).

Color

Gelatin color measurements were carried out using a Chromameter (Konika Minolta Sensing, INC, Japan) carried out with the Hunter system, expressed in L*, a* and b* values. L* (brightness) value for light are in the range of 100 = white and 0 = black. Values of a* (reddish) are in the range of -50 (green) and +50 (red). While the value of b* (yellowish) is in the range of -50 (blue) and +50 (yellow).

RESULTS AND DISCUSSION

Gel Strength

Gel strength is the most important aspect in determining gelatin quality (Mulyani *al*., 2017). The strength values of the gel on goat skin gelatin shown in table 1 have met the ISO 75-300 g bloom (Sultana *et al*., 2018) standard for general industry use. Statistical analysis showed a very significant effect ($P<0.01$) on age and acid pretreatment, as well as interactions between goat age and pretreatment of acid sources (*L. plantarum* and acetic acid), tendency to react (figure 1) at age 1 year.

Table 1. Gel Strength of goat skin gelatin with *L. Plantarum* 1UHCC and Acetic Acid (CH_3COOH) pretreatment

Goat Age (year)	Gel Strength (g bloom)		Average
	L. Plantarum	Acetic Acid	
1	146.23±0.22	121.58±1.09	$133.91±0.66^{az}$
2	120.48±0.26	69.38±0.15	$94.93±0.21^{bz}$
3	91.14±0.5	49.41±0.33	$70.27±0.42^{cz}$
Average	$119.28±0.33^{pz}$	$80.12±0.52^{qz}$	

Note: ^{abcpq} superscripts in the same row and column show significant differences (P<0.05) and (P<0.01)

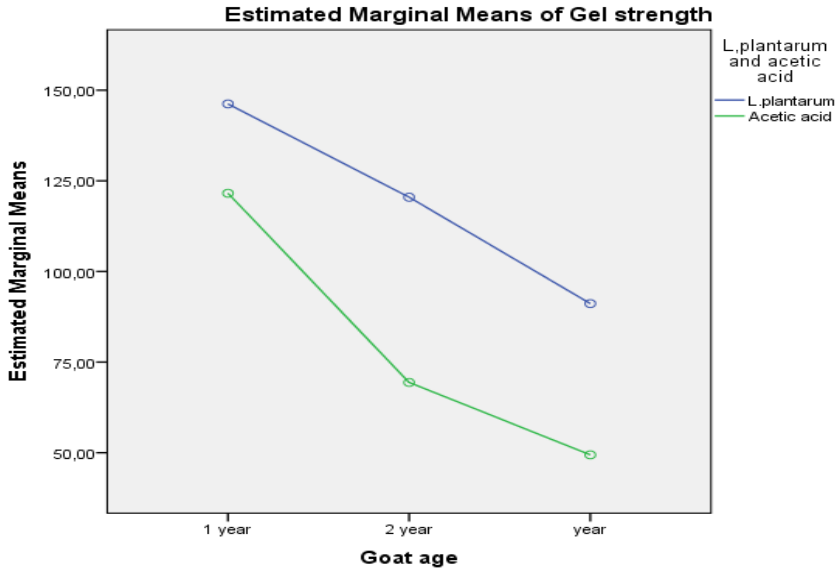

Figure 1. Interaction between acid source and goat age on gel strength

Table 1. the gel strength value of 1 year old (133.91±0.66 g bloom) showed a significant increase compared to the age of 2 years (94.93±0.21 g bloom) and 3 years (70.27±0.42 g bloom). Skin connective tissue at the age of 1 year is not compact compared to ages 2 and 3 years so that it will facilitate the breaking of the triple helix protein double bonds in acid pretreatment. Arnesen & Gildberg (2002) reported that gel strength is highly dependent on hydrogen bonding between free hydroxyl groups and water molecules from amino acid groups, concentration, protein chain size and collagen molecular distribution.

L. plantarum pretreatment showed a higher gel strength value (119.28±0.33 g bloom) than acetic acid pretreatment (80.12±0.52 g bloom). The increase in the gel strength value with *L. plantarum* pretreatment occurs because the lactic acid produced is able to break the polymer chain of amino acids more precisely than acetic acid. In line with the research of (Said *et al.*, 2011) that by providing curing materials will work to break the polymer chain of amino acids at the right and optimum limits, so that it will provide an effect of improvement in the process of gel formation. Furthermore, the monomer chains of amino acids combine with each other to form a three-dimensional structure continuously and bind water to form a compact gel.

Figure 1. The direct interaction between acid pretreatment (*L.plantarum* 1UHCC, Acetic acid (CH_3COOH)) and the age of the goat to the strength of the gel showed different response

under the same conditions. At the age goats of 1 year have a high response to the strength of the gel, but at the age of 2 and 3 years with acid pretreatment (*L.plantarum* 1UHCC, acetic acid (CH_3COOH) tends to decrease. So it can be said that the age of 1 year shows effective results. High gel strength values are able to effectively convert collagen to gelatin so that the size of the hydrolyzed collagen gelatin peptide chain is longer than other pretreatments (Dewi & Hasdar, 2017).

Acidity (pH)

The acidity (pH) greatly influences the chemical properties of gelatin (Ratna *et al*., 2017) because it is related to viscosity, water binding ability and emulsion capacity (Abustam *et al.*, 2008).

Table 2.pH of goat skin gelatin with *L. plantarum* and Acetic Acid (CH_3COOH) pretreatment

Goat Age (year)	pH		Average
	L. Plantarum	Acetic Acid	
1	5.24±0.16	5.25±0.18	$5.25±0.17^{a}$
2	5.56±0.18	5.56±0.18	$5.56±0.18^{b}$
3	5.65±0.17	5.65±0.14	$5.65±0.16^{b}$
Average	5.48±0.17	5.49±0.17	

Note: ^{abc} superscripts in the same column show significant differences (P<0.05) and (P<0.01)

Based on the statistical analysis in Table 2. The difference in goat age shows a significant effect (P<0.05) on pH, but does not affect the pretreatment of acid given (P>0.05). The pH value at the age of 1 year goat 5.25±0.17 shows the difference with the age of 2 and 3 years, respectively 5.56±0.18 and 5.65±0.16. The results obtained are considered very good because it does not affect the acid pretreatment given during the production process. Said et *al*. (2011) reported that the condition of the pH value in the neutral range indicated that the process of neutralizing or washing raw materials before extraction went perfectly.

The pH value of goat skin gelatin obtained 5.25±0.17-5.65±0.16 pH of the goat skin gelatine (Sultana *et al*., 2018) ranging from 3.8 to 7.5.

Viscosity

Viscosity or thickness of gelatin is the ability to hold a liquid. (Haug and Draget, 2009)reported that viscosity occurs due to the development of colloids and adsorption from hydrocolloid products.

Table 3. Viscosity of goat skin gelatin with *L. plantarum* and Acetic Acid (CH_3COOH) pretreatment

Goat age (year)	Viscosity (cP)		Average
	L. Plantarum	Acetic Acid	
1	9.22±0.09	7.61±0.12	$8.42±0.11^{a}$
2	8.64±0.38	7.49±0.08	$8.07±0.23^{b}$
3	7.37±0.33	6.09±0.08	$6.73±0.21^{c}$
Average	$8.41±0.27^{p}$	$7.06±0.09^{q}$	

Note: ^{abcpq} superscripts in the same row and column show significant differences (P<0.05) and (P<0.01)

The age of the goats and different acid pretreatment according to Table 3, give results a very significant effect (P<0.01) but there was no interaction between the two. Viscosity values at different ages ranged from 6.73±0.21cP to 8.42 ± 0.11 cP. The age of the goat is closely related to the stability of collagen. (Juliasti *et al.*, 2014) reported that aging increases the stability of collagen and causes an increase in the amount of cross-linking in collagen.

Acid pretreatment ranged from 7.06±0.09 cP to 8.41±0.27 cP. The viscosity value obtained is still within the ISO threshold range of 2.0-7.5 cP (Ulfah *et al.*, 2011). The difference in viscosity is influenced by the molecular structure of the amino acids making up gelatin protein (Said *et al.*, 2011a). The longer the amino acid composition, the higher the viscosity of gelatin (Said *et al.*, 2011b).

Color

Gelatin colors show physical characteristics related to aesthetics, bright colors tend to be preferred when mixed into certain food products (Shyni *et.al.*, 2014). The results of the measurement of goat skin gelatin color brightness L* can be seen in Table 4a.

Table 4a. Brighhtness color L* goat skin gelatin with *L. plantarum*1UHCC and Acetic Acid (CH_3COOH) pretreatment

Goat age (year)	Measurement of Color L* (%)		Average
	L. Plantarum	Acetic Acid	
1	64.68±0.52	62.77±1.18	$63.73±0.85^{az}$
2	69.52±2.29	74.11±0.34	$71.82±1.32^{bz}$
3	68.29±1.57	76.38±1.10	$72.34±1.34^{bz}$
Average	$67.50±1.46^{pz}$	$71.09±0.87^{pz}$	

Note: ^{abpq} superscripts in the same row and column show significant differences (P<0.05) and (P<0.01)

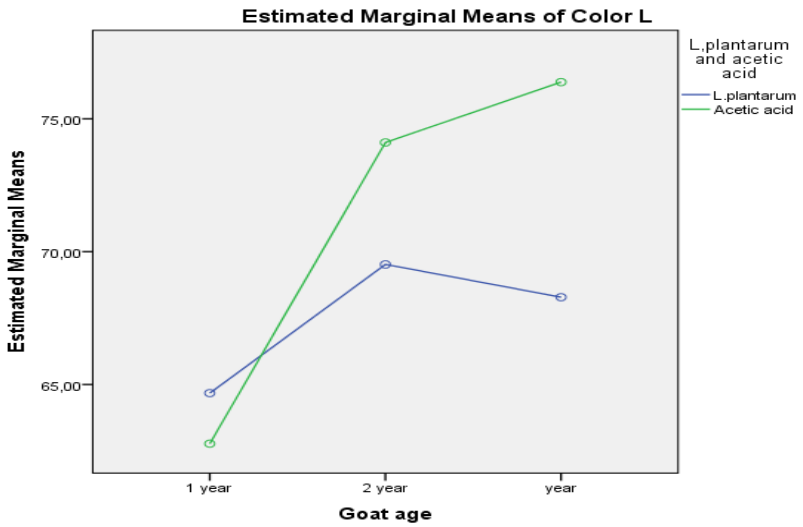

Figure 2. Interaction between sour sources and goat age with brightness (L*)

Table 4a. shows the level of brightness L* very significant effect (P<0.01) on goat age and acid pretreatment. The higher the age of the goat, the brightness level increased by 72.34±1.34 %, as well as the value of acetic acid pretreatment was higher (71.09±0.87%) compared to *L. plantarum* 1UHCC 67.50±1.46 %. Color change affects the administration of acid which results in the loss of pigment characterized by the release of cell fluid during the extraction process (Shyni *et al*., 2014).

Figure 2. Shows the effect of the interaction between acid pretreatment (*L. plantarum* 1UHCC and acetic acid (CH_3COOH) with goat age on the brightness color (L*) in the age range of goats 1 to 2 years. Acetic acid pretreatment (CH_3COOH) has sensitivity to goat age because it has increased brightness L* compared to *L.plantarum* which shows an increase in brightness L* at 2 years but 3 years of age tends to decrease.

Table 4b. Reddish color a* goat skin gelatin with *L. plantarum* 1UHCC and Acetic Acid (CH_3COOH) pretreatment

Goat age (year)	Measurement of Color a* (%)		Average
	L. Plantarum	Acetic Acid	
1	10.32±0.57	9.7±0.62	$10.01±0.60^{a}$
2	10.88±1.14	6.99±1.30	$8.94±1.22^{a}$
3	11.13±1.58	5.91±2.07	$8.52±183^{a}$
Average	$10.78±1.10^{p}$	$7.53±1.33^{p}$	

Note: ^{ap} superscripts in the same row and column show significant differences (P<0.05) and (P<0.01)

The results of statistical analysis of reddish color a* goat skin gelatin in Table 4b. showing a significant effect (P<0.05) on goat age and acid pretreatment, although it did not show differences between the two the highest reddish a* at the age of 1 year was 10.01±0.60 % with *L. plantarum* pretreatment 10.78±1.10 %.

L. plantarum which produces lactic acid is still able to maintain the miyoglobin pigment on the skin of goats. The potential for color degradation at the age of 3 years of goats is due to the reduction in miyoglobin pigment. a* (reddish) color reduction is still within reasonable

threshold. opinion Magdic *etal.* (2009) on the Hunter system a* (reddish) color ranges between 2-30%.

Table 4c. Yellowish color b* goat skin gelatin with *L. plantarum* 1UHCC and Acetic Acid (CH_3COOH) pretreatment

Goat Age (year)	Measurement of Color b* (%)		Average
	L. Plantarum	Acetic Acid	
1	31.65±0.88	30.26±0.64	30.96±0.76
2	33.81±2.34	27.65±0.20	30.73±1.27
3	31.21±1.25	28.83±1.13	30.02±1.19
Average	$32.22±1.49^p$	$28.91±0.67^p$	

Note: ^{p} superscripts in the same row show significant differences (P<0.05)

While the yellowish color b*showing a significant effect (P<0.05) on acid administration but no effect on goat age.The highest yellowish value b* in *L. plantarum* pretreatment 32.22±1.49 %.

The higher the color value b* (yellowish), the more yellowish. Magdic *etal.,* (2007). This is influenced by amino groups that react to the skin carbonyl gelatin compounds will produce a non-enzymatic browning reaction, thus causing an increase in yellowing of gelatin.

CONCLUSION

The age of the goat and the administration of different acid pretreatments had an impact on the physicochemical properties of goat skin gelatin. One-year-old goat with *L .plantarum* pretreatment showed optimal results in physicochemical properties of Goat skin.

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