Effectiveness of *Lactobacillus plantarum* 1UHCC bacteria in partial hydrolysis of goat bone in producing gelatin

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**Abstract.** Gelatin was obtained from the bones of goats that are hydrolyzed in several stages. In the initial stage, immersion is carried out using an acid solution so that the goat’s bones can be partially hydrolyzed and the connective tissue of the bone becomes loose so that the gelatin can be easily extracted. Partial hydrolysis in goat bones using chemical acids has been widely used but very few utilize biological acids produced by Lactic Acid Bacteria (BAL), specifically *Lactobacillus plantarum*. Therefore, it is necessary to grow lactic acid bacteria in maintenance media to produce lactic acid as a preservative in the bones. This study aimed to obtain preliminary information to determine the ability of *L. Plantarum* 1UHCC to degrade goat bone protein soaked in MRSB media. The indicators measured were lactic acid, pH, total bacteria, and total dissolved protein with different soaking times. This was conducted according to a completely randomized design (CRD) with three treatment duration of fermentation (24, 48 and 72 h, repsectively) and three replications. The results showed that there was a significant effect (P<0.01) of the fermentation time on lactic acid, pH, total bacteria, dissolved protein value. Lactic acid concentration at 24 h fermentation time was 0.95±0.01% and this values was decreased to 0.82±0.092% after fermented for 48 h, but showed a significant increase at 72 h (0.942±0.02%). The pH value at 24-hour incubation were 5.41±0.015 then increased to 6.60±0.044 at 48 h of incubation; and decreased to at 72 h 6.30±0.266 at 72 h. Total bacterial values showed an increase in fermentation time of 24 h, 48 h and 72 h respectively were 8.248±0.196 ; 9.475±0.274 ; 9666±0.236 (log10CFU/mL). The value of dissolved protein showed an increase of 31.170±0.120 mg/mL; 31.183±0.101 mg/mL; 32.707±0.115mg / mL. This study indicated that 72 h fermentation time showed better quality of partial hydrolysis in goat bones.

1. **Introduction**

Goat bones contain collagen, a bone protein and as a basis for making gelatin. According to Hermanianto [1], the gelatin food industry has many uses including as a binder agent, adhesive, gelling agent, whipping agent, viscosity agent, thickener, and stabilizer. Gelatin production can be divided in several stages, the initial stage is soaking using an acid solution [2] with to obtain partially hydrolyzed goat bones, the connective tissue becomes loose so that the gelatin is easily extracted. The process of partial hydrolysis of goat bones using chemical acids, bases and enzymes has been widely carried out, but still very little studied using biological acids with the utilization of Lactic Acid (BAL)
Lactobacillus plantarum 1UHCC culture. The use of L. plantarum 1UHCC in the process of making healthy gelatin will be a solution to get safe products and environmentally friendly.

The addition of L. Plantarum 1UHCC in the fermentation media aims to break down the protein in the bones, that the bacteria need to grow on the growth medium. This research was preliminary information to determine the ability to grow L. Plantarum 1UHCC and to evaluate partial hydrolysis of goat bones with indicators of lactic acid levels, pH, total bacteria, and total dissolved proteins. The treatment of this study was done by different soaking times.

2. Materials and methods

2.1. Materials

The material was used 1 year old Ettawah Peranakan (PE) male goat skin which had been cleaned with fur and fat, BAL L. Plantarum 1UHCC strain 1UHCC 5% concentration, MRS-agar, MRS-broth, NaOH 0.1N, PP (phenolphthalein ), aquades, buffered pH4 and pH7.

2.2. Methods

This study uses a Completely Randomized Design 3x3 with 24 hours, 48 hours and 72 hours fermentation treatment each time repeated 3 times. Lactic Acid levels has been used titration method [3], using 0.1N NaOH and PP (phenolphthalein). pH has been measured using potentiometric methods[3], the pH meter which was first calibrated using a buffer of pH 4 and pH 7. Total Bacteria has been counted with a counter colony, and has used pouring method [4], using MRS-agar media and plate count as a means of calculating total bacteria. Total protein dissolved method [5] detected by Lowry Methods that adding lowry A (Follin-Ciocalteus and aquades ratio 1:1), lawry B (2% Na2CO3 /NaOH 0.1N, 1% CuSO4, Na-K- tartrate 1% 100:1:1 ratio) for each sample.

3. Results and discussion

3.1. Lactic acid levels

The effect of fermentation time on lactic acid fermentation of the medium culture contain goat bone by L. plantarum fermentation presented on Table 1. The results of the analysis variance of lactic acid levels in response to the use of L. plantarum 1UHCC and different fermentation times showed very significant differences effect (P<0.01).

Lactic acid levels was increased at 24 hours fermentation time (0.954±0.001%), but was decreased at 48 hours fermentation (0.821±0.092%), then increased again at 72 hours fermentation (0.943 ±0.002%). The value of lactic acid obtained was higher compared before research [6] which obtained 0.92% lactic acid in 60 hours fermentation time. Decreasing the lactic acid value in fermentation for 48 hours, due to of L. plantarum 1UHCC ability to produce lactic acid. The decreasing of the nutrients contained in MRS-broth have cause of L. plantarum 1UHCC take advantage of goat bones to be overhauled as a source of nutrients in maintain its life. Aruni et al [7] suggested that L. plantarum was able to convert protein compounds into simple compounds so that they were able to produce lactic acid and the reform was called glycoprotein reshuffle.

Table 1. Characteristics of L. plantarum 1UHCC culture on PE goat bones media at different fermentation time

| Parameter        | 24          | 48          | 72          | Average     |
|------------------|-------------|-------------|-------------|-------------|
| Lactic acid      | 0.954±0.001 | 0.821±0.092 | 0.943 ±0.002 | 0.906±0.032 |
| pH               | 5.413±0.015 | 6.600±0.044 | 6.303±0.266 | 6.106±0.108 |
| Total bacteria   | 8.248±0.196 | 9.475±0.274 | 9.666±0.236 | 9.129±0.235 |
| Dissolved protein| 31.170±0.120| 31.183±0.101| 32.707±0.115| 31.687±0.112|

aDifferent superscripts in the same row showed a significant difference (P<0.01)
3.2 pH
The results of the pH analysis (Table 1) have a significant effect (P<0.01) on fermentation time of goat bones. The 24 hour fermentation was more acidic (5.413±0.015) than 48 hours fermentation (6.600±0.044), but it has increase the acidity in 72 hours fermentation (6.303±0.266). L. plantarum 1UHCC has degraded goat bone glycoprotein to lactic acid thereby reducing pH. Chelule et al [8] reported that the decrease in pH occurred due to the production of lactic acid from L. plantarum.

Figure 1. presented the peak point in the fermentation period 48 hours and then has decreased in 72 hours fermentation, [9] the decrease in pH value is marked by the increase in fermentation time.

3.3 Total bacteria
Total bacterial analysis in Table 1. was resulted a very significant effect (P<0.01) in 24-hour fermentation time (8.248±0.196 log10 CFU/mL). The increase in total bacteria occurred in 48 hours fermentation (9.475±0.274 log10 CFU/mL) as wellm 72 hours fermentation (9.666±0.236b log10 CFU/mL). Availability of nutrients to the environment will increase the productivity of L. plantarum during fermentation so that total microbes will increase [4].

Figure 1. presented the 72 hour fermentation that increasing growth of L. plantarum 1UHCC. The same study[10], within 72 hours of silage fermentation with the addition of L. plantarum resulted very significant increase of cell growth. This condition still allows microbes to grow due to [11] adequacy of nutrients that used for the growth and development of microbial cells.
3.4 Dissolved protein
The dissolved protein analysis in Table 1. was resulted a very significant effect (P<0.01) in the 24 hour fermentation time (31.170±0.120 % g/mL), but there is no difference with 48 hours fermentation (31.183±0.101 % g/mL), and there was difference in 72 hour fermentation (32.07±0.115% g/mL). The L. plantarum fermentation process grows along with the availability of nutritional sources [8]. This is cause of [12] protein has maximum absorption in the presence of peptide bonds. This study is in line with [13] has been reportet that fermentation with the addition of L. plantarum starter culture will be gradated of protein by the amount of proteolytic degradation from the first day of 5.19%, was increased to 23.33% on the seventh day of fermentation. The amount of proteolytic degradation in the fermentation process with the addition of a L. Plantarum 1UHCC starter culture is thought to be the presence of a proteolytic enzyme system from L. plantarum B1765 that is able to degrade proteins into dissolved proteins, peptides, or amino acids.

Figure 1 illustrated the longer the fermentation, and the higher the level of dissolved protein. This shows the integration of some dissolved proteins in the surrounding environment. One of the desired effects on the growth of L plantarum is the acidification process that occurs where L plantarum hydrolyzes carbohydrates and proteins into lactic acid [14].

4. Conclusion
There was a significant difference effect between each levels of lactic acid, pH, total bacteria, dissolved protein in medium that contain goat bone by added L. plantarum at different fermentation time. The increase of fermentation time, resulted the increase of L plantarum 1UHCC growth, lactic acid, and dissolved protein, otherwise with pH.

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