Potential of Goat Whey Added with Soy Milk as Alternative Growth Media for *Lactobacillus plantarum*

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ABSTRACT
A study on the potential of goat whey as a growth medium for lactic acid bacteria has been carried out at the Laboratory of Science and Technology of Milk Processing, Faculty of Agriculture, University of Syiah Kuala Banda Aceh, Indonesia. The purpose of this study was to examine the performance of goat whey as a growth medium after being added with soy milk, both as liquid and agar media to the growth of *Lactobacillus plantarum*. This study used a completely randomized design (CRD) consisting of 5 (five) treatments and 4 replications. The treatments given are goat milk whey as basal media (control); basal media+1.5% soy milk; basal media+3% soy milk; basal media + 4.5% soy milk and basal media + 6% soy milk, so 20 experimental units were obtained. Parameters measured were pH, protein content, lactic acid content and total plate count of *Lactobacillus plantarum*. Furthermore, the data obtained were analyzed using ANOVA (Analysis of Variance), and if there were differences between treatments, it would be followed by Duncan's Multiple Range Test (DMRT). The results showed that the addition of soy milk had a significant effect ($P<0.05$) on protein content and a very significant effect ($P<0.01$) on pH values and lactic acid levels. Bacterial growth occurred was characterized by an acidic aroma on the petri dish because the colonies were countless due to the turbidity of the solid media after sterilization. The best alternative media was obtained from the addition of 4.5% soy milk with the results of pH, protein content, and lactic acid content respectively were 4.17, 2.83, and 0.59.

Keywords: *whey, soy milk, viability, liquid media, lactic acid.*

1. INTRODUCTION

Goat whey is a clear liquid that is separated from solids produced by fermenting milk or cheese-making process which still has a fairly complete nutritional content and is widely used in the food industry as a functional food [1] It is composed of approximately 0.3% fat, 0.8% protein, 4.9% lactose, and 0.5% minerals. Considering the nutrient contents, goat whey could be used as culture media for the growth of lactic acid bacteria (LAB) as an alternative medium to commercial ones. Based on another study it was found that whey contains only 0.76% protein and other nutrients [2]. These results indicate that with such nutritional value whey can be used as an alternative medium for growing LAB. One of these lactic acid bacteria is *Lactobacillus plantarum*. This bacterium is a LAB that is often found in food and feed with high nutritional content and has very high adaptability. This indicates that *L. plantarum* can utilize carbohydrates and other compounds considering its final metabolite is lactic acid [3].

As an alternative medium due to the low protein content of goat whey, it is necessary to add other nitrogen sources to meet the nutritional adequacy, in this case, nitrogen that comes from soy milk. The protein content in soy milk represents vegetable protein which contains almost 4% of its dry weight [4]. Soy milk is a good media for LAB growth because it contains oligosaccharides (*sucrose, raffinose, and stachyose*) so that it can be used as an energy source [5]. According to the Indonesian National Standard (SNI), soy milk is a product derived from soybean seed extract which contains nutrients in the form of fiber, unsaturated fatty

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acids, vitamins, and minerals, as well as high protein content ranging from 1.16-2.04%. Growth media containing optimal soy protein has a beneficial effect on the growth of *L. plantarum* [6]. This study aimed to study the potential of whey as a growth media for *L plantarum* after the addition of soy milk.

2. MATERIALS AND METHODS

2.1. Material

The materials used in this research were goat's milk which was obtained from UD Abi Makmur Sentosa, rennet (QSO), NaCl, *Lactobacillus plantarum* FNCC 0027, liquid soybean product, NaOH 0.1 N, buffer solution at pH 4 and 7, PP (phenolphthalein) 1% indicator, potassium oxalate solution, formaldehyde solution, filter cloth, tissue, label paper, aluminium foil, cotton, and alcohol 70%.

2.2. Method

2.2.1. Starter preparation

At this stage, 250 mL of fresh goat's milk was pasteurized at 85°C for 15 sec. Then, 5% pure culture of *Lactobacillus plantarum* was inoculated into pasteurized milk and incubated at 37°C for 18 h to get a starter that would be grown on cheese whey growth media.

2.2.2. Goat Whey preparation

The whey used in the current study was collected from cheese making according to the method proposed by [7]. Fresh goat milk was poured into a pan, then pasteurized at 72-74°C for 15 sec. Pasteurized goat milk was then left until it reached a temperature of 30-45°C, then added with 0.025% *rennet* that previously has been dissolved in water, and left until the milk clots process occurs and forms a *curd*. Furthermore, the formed curd was cut into pieces to make the whey separation easier, then left for a few hours to the *curd* and whey separate completely.

2.2.3 Media preparation

2.2.3.1 Liquid media.

Pasteurized goat whey used as a basal medium was added with soy milk based on the designed treatment, namely goat whey without soy milk as control; goat whey + 1.5% soy milk: goat whey + 3% soy milk; goat whey + 4.5 % soy milk and goat whey + 6% soy milk, each treatment was repeated 4 times. Then each sample was inoculated with 5% starter culture of *L. plantarum*, homogenized and then incubated at 37 ^{o}C for 18 h. The next stage is testing the protein content, pH, lactic acid content and the total number of bacteria of each sample of treatments

2.2.3.2 Agar media.

In this preparation, 20 g of agar was added to 1000 mL of pasteurized goat whey as a basal medium, heated using a hot plate for homogenization. It was then sterilized using an autoclave at 121°C for 15 min (agar media), and cooled to 40°C.

2.2.4. Protein level test

At this stage, the protein content was tested using the formol titration method as proposed by [8]. As much as 10 mL of the sample was put into an Erlenmeyer, added with 0.4 mL of potassium oxalate solution and 0.5 mL of phenolphthalein solution, and then left for 2 min. Furthermore, the sample was titrated using NaOH 0.1 N until it became pink. After that, 2 mL of formaldehyde solution was added and homogenized, left for 1 minute. The sample was further titrated again using NaOH 0.1 N until it became pink as well. In this case, the volume of NaOH solution used in the second titration was recorded. Furthermore, 10 mL of distilled water as blank was put into an Erlenmeyer, and added with 0.4 mL of potassium oxalate and 0.5 mL of phenolphthalein solution, left for 2 minutes. The blank was further titrated using NaOH 0.1 N until it became pink, then added with 2 mL of formaldehyde solution and homogenized, left for 1 min. After that, the blank was titrated again using NaOH 0.1 N until it became pink, and the solution volume of NaOH 0.1 N in the second titration was recorded. Whey protein levels are calculated using the following formula:

$$(\text{mL NaOH sample} - \text{mL NaOH blank}) x\ 1{,}95$$

2.2.5. Acidity test (pH)

Measurement of pH was carried out using a pH meter according to the method proposed by [9]. The pH meter was calibrated first with a buffer solution of pH 4 and 7. Then, the electrode was washed using distilled water and dried. The pH of the sample was further measured by dipping the pH meter electrode into 10 ml of the sample and the numbers listed on the monitor were recorded. This process was carried out for all sample units.

2.2.6. Lactic acid test

The lactic acid level was measured using the method proposed by [10]. The burette was filled with NaOH 0.1 N solution slowly so that no air bubbles were formed. As much as 18 mL of whey was weighed in a beaker glass, then 3-4 drops of 1% phenolphthalein indicator were added. Furthermore, the titration was carried out using NaOH 0.1 N until the color change was formed

and the volume of titer used was calculated. Lactic acid levels are calculated using the following formula:

$$\text{Lactic acid level} = \frac{\text{ml NaOH} \times \text{N} \times 90}{\text{Sample weight (g)} \times 1000} \times 100\%$$

2.2.7. Total bacteria count

Serial dilutions were carried out in the calculation of total bacteria. As much as 1 mL of starter *L. plantarum* was transferred into 9 ml of diluent (10^{-1}), then this step was carried out until the dilution of 10^{-6}. Furthermore, 1 mL of the 10^{-6} dilution was pipetted into a petri dish in duplo, then 15 mL agar media was poured into it, homogenized and incubated for 24 h at 37°C. This procedure was carried out for each treatment that has been set. The total bacteria were further expressed in log CFU/mL, using the following formula:

$$\text{CFU/mL} = \text{Number of colony} \times \frac{1}{\text{Diluent factor}}$$

2.2.8. Statistical analysis

The experiment was a completely randomized design with five treatments and three replications. The treatments consisted of different levels of soy milk, namely 0%, 1,5%, 3%, 4,5% and 6%. The parameters measured were pH, protein, lactic acid level, and a total of lactic acid bacteria (LAB). Data were analyzed by using ANOVA, if there is a treatment effect, it will be continued with Duncan's further test to see the difference between treatments [11].

3. RESULTS AND DISCUSSION

Based on table 1, it can be seen that the addition of soy milk at different levels in goat whey as a basal media had a significant effect (P<0.05) on protein levels, pH and lactic acid levels.

Table 1 show that the increase in protein content was in line with the addition of soy milk. The higher the percentage of added soy products, the more protein content produced. This is caused by the growth of the lactic acid bacteria (LAB) which utilize protein as a source of nutrients in cell metabolism. In addition, Lactic acid bacteria also use carbon as an energy source and nitrogen source in their growth [12]. During the fermentation process, lactic acid bacteria will secrete several enzymes, one of them is proteolytic enzymes. Proteolytic enzymes will help break down proteins and facilitate the penetration of lactic acid bacteria into casein micelles, this causes changes in protein level in fermented milk products [13][14].

Furthermore, the protein content in the fermented product is the total amount of the protein material used and the protein of lactic acid bacteria contained [15]. The difference in protein levels indicates that *L. plantarum* has grown in the liquid media.

Changes in pH during the fermentation process indicate that there is microorganism activity, in this case, is *Lactobacillus plantarum*. Based on Table 1, the addition of soy milk had a very significant effect (P<0.01) on the pH of goat whey liquid media, it was seen that there were differences between treatments. The more soy milk added, the lower the pH of the medium. This happened until the addition of 4.5% soy milk but did not differ from the addition of 6%. The decrease in pH indicates that the addition of 4.5% is the maximum level of carbohydrate sources that can be fermented by *L. plantarum* into lactic acid so that any increase in the level of soy milk will not lower the pH.

The decrease in pH in each treatment showed that the metabolism of lactose into lactic acid and the carbohydrate content of soy products had occurred optimally. The use of soy products in goat whey aims to provide adequate nutrients for the growth of *L. plantarum*, such as carbohydrates, protein, calcium, phosphorus, sodium, fat, riboflavin, niacin, iron, thiamin, and sodium. Therefore, the decrease in pH proves that there has been an optimal fermentation process by *L. plantarum.*

Based on Table 1, shows that the addition of soy products significantly (P<0.05) affected the lactic acid level. In this case, the addition of soy milk by 1.5 and 3% did not show any difference. However, there was a significant difference found between the addition of 4.5 and 6%. The lowest lactic acid level found in the control was 0.46 (goat whey without BAL) This value indicates the occurrence of carbohydrate metabolism by *L. plantarum*, because basically goat whey still contains

Table 1. Average of Whey composition as growth media of *L. plantarum*

Parameter	Level of soy product (%)				
	0	1.5	3	4.5	6
Protein level	2.63 ± 0.12^{ab}	2.58 ± 1.01^{a}	2.68 ± 0.19^{abc}	2.83 ± 0.11^{bc}	2.87 ± 0.10^{d}
pH	4.42 ± 0.02^{d}	4.30 ± 0.01^{c}	4.20 ± 0.01^{b}	4.17 ± 0.01^{a}	4.17 ± 0.10^{a}
Lactic acid level	0.46 ± 0.01^{a}	0.52 ± 1.01^{b}	0.52 ± 0.01^{b}	0.59 ± 0.01^{c}	0.61 ± 0.01^{d}
TPC CFU/ml	+	+	+	+	+

Note: the mean of protein with different superscripts in rows and columns is significantly different (P<0.05) of protein, the mean of pH (P<0,01), (+): growth of *L. plantarum* (too much to be counted)

4.9% lactose [1]. Lactic acid levels were found to be higher compared to the control but there was no difference in the treatment of 1.5 and 3% addition of soy milk. This explains that *L. plantarum* can digest soy milk carbohydrates in the form of sucrose, raffinose, and stachyose as a source of energy for growth [3]. It can be seen that the higher the level of addition of soy milk, the more carbohydrate are converted into lactic acid and other organic acids. This explains that *L. plantarum* optimally performs its metabolism by using carbon sources in its growth. Based on a study using deproteinized whey that the addition of yeast extract as a nutritional source has increased not only the degree of acidification by *Lactobacillus bulgaricus* 11842 but also the activity of -galactosidase. This activation leads to lactose degradation and good glucose uptake which results in good growth [16]. Based on a study using deproteinized whey that the addition of yeast extract as a nutritional source has increased not only the degree of acidification by *Lactobacillus bulgaricus* 11842 but also the activity of -galactosidase. This activation leads to lactose degradation and good glucose uptake which results in good growth.

Furthermore, the fermentation process by LAB is characterized by an increase in organic acids, which are lactic acid, malic acid, and acetic acid. Such condition indicates that LAB can grow and function properly when it receives adequate nutrition. The more nutrients used by LAB during fermentation, the more organic acids, and other metabolite products produced [17]. LAB utilizes sugar as an energy source for growth and produces metabolites in the form of lactic acid in the fermentation process which causes an increase in the breakdown of sugar compounds contained in the media into organic acids. Furthermore, the production of lactic acid by bacteria is determined by several factors, including the ability of bacteria to utilize carbon sources contained in the fermentation media as well as the type, source, and amount of sugar [18].

Based on the total bacterial count, *L. plantarum* cells grown on goat whey agar media could not be counted due to turbidity in agar media. This may be due to the goat whey used as the basal medium not being deproteinized. As an indicator of the occurrence of growth is the presence of sour aroma produced by the growth of *L. plantarum*, in addition to changes in protein levels, decreased pH and lactic acid levels in liquid goat whey media.

The results of [5] research obtained that the growth pattern of *L. plantarum* in fermented soy milk increased during the fermentation period. Another study conducted by [19] found that the estimated optimum number of medium factors for the growth of *L. plantarum* jnu 2116 one of which is the content of soy-peptone as much as 0.213% glucose.

4. CONCLUSION

In conclusion, goat whey has potential as an alternative medium for the growth of *L. plantarum*, it can be seen from changes in pH, protein content, and lactic acid levels during the fermentation process in goat whey liquid media. However, the total LAB which cannot be calculated on goat whey agar media proves that goat whey must be treated before being used as an agar medium. Optimal growth was obtained with the addition of 4.5% soy milk.

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