The effect of additional skim and fermentation time on the amount of lactic acid bacteria and the pH of soyghurt

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Abstract. This study aimed to determine the effect of 5% skim and fermentation time on the number of lactic acid bacteria (LAB) of soyghurt. This study used completely randomized factorial design with 2 factors: skim addition and fermentation time. Standard soyghurt consisted of soybean juice, granulated sugar (5%), and starter (2%) containing Lactobacillus acidophilus, Lactobacillus bulgaricus, and Streptococcus thermophilus, fermented at 37°C for 10, 12, and 14 h. LAB were cultured in deMann Rogossa and Sharp (MRS) medium and then calculated by total plate count. The number of LAB in soyghurt with 5% skim addition is higher (1.76 x 10^8 CFU/ml) than soyghurt without 5% skim addition (5.9 x 10^6 CFU/ml). The number of LAB increased along with the length of fermentation. However, a significant difference in the average number of soyghurt lactic acid bacteria (p >0.05) are not found in 10 h, 12 h, and 14 h fermentation in both treatments. The longer fermentation time indicated that the pH of soyghurt was smaller. These results suggested that the addition of 5% skim and 14 h fermentation resulted in soyghurt with the highest number of LAB with pH of 3.25.

1. Introduction
Soyghurt is soybean juice fermented by adding lactic acid bacteria which allows coagulation of soy protein and makes it a kind of yoghurt [1]. Protein in soybean extract can boost the growth of probiotic bacteria, such as Lactobacillus acidophilus, Lactobacillus casei, and Streptococcus salivarius [2]. During their growth, lactic acid bacteria will slowly release protons and allow a decrease in pH which lets soy milk protein gradually coagulated to form aggregates [1]. The use of lactic acid bacteria plays important role in the improving of human health such as regulate the cholesterol level in the blood, improve the lactose metabolism and improving the digestive disease. Lactic acid bacteria have an ability to survive in the human colon and then regulate the number of colon flora and also boost the immune system [1,2].

The benefits of soybean as food is in line with the concept of national functional food due to the isoflavone compound and other beneficial compound that can be used as treatment for degenerative disease. Based on dry weight, soybean contains about 40% protein, 20% oil, 35% soluble carbohydrates (sucrose, stachyose, raffinose, etc.) and insoluble carbohydrates (dietary fibre), and 5% ash [3]. The types of carbohydrates in soybean juice are highly different from those in cow's milk. Carbohydrates in soymilk consist of oligosaccharides. Soybean can be processed to soy milk which then be processed into soyghurt with a high protein value. Soyghurt is lactose free so that can be consumed by people who are...
lactose intolerant. Soyghurt has a lower level of cholesterol, saturated fatty acids and lactose compared to yoghurt. However, the taste of soyghurt still exhibits beany flavor so that Indonesian people rarely consume it [4].

The factors which may affect the growth and survival of lactic acid bacteria are chemical composition and nutrient content. Lactic acid bacteria need enough amino acids and carbohydrates for growth. Skim milk has high protein and lactose content, usually used to increase the growth of lactic acid bacteria. Lactose is a carbohydrate in skim and is a substrate which can be fermented by lactic acid bacteria [5]. In addition, the bacteria will grow very quickly when it reaches the logarithmic phase, so soyghurt can be obtained with the optimal amount of lactic acid bacteria. It needs the right fermentation time. Nowadays, there is a limited research about the use of soyghurt as functional food and synbiotic source. Thus, this study aimed to determine the effect of 5% skim and fermentation time on the number of lactic acid bacteria (LAB) of soyghurt.

2. Methods

2.1. Materials
This study used soybean devon 1 variety, fresh cow milk, and starter with lactic acid bacteria compound (Lactobacillus acidophilus, Lactobacillus bulgaricus dan Streptococcus thermophilus). The materials used in this study were beaker glass 250 ml, glass basin, spatula, thermometer, refrigerator, incubator, stove, pan, and analytic scale.

2.2. Research design
This study is an experimental laboratory using a factorial design method with 2 factors, the addition of skim (standard, 5% skim) and fermentation time (10 hours, 12 hours and 14 hours). Each treatment is performed 3 times, so 18 experimental units are obtained.

2.3. Soybean juice preparation
Soymilk is made by soaking soybean for 8 hours, then washing it until it is clean. Afterwards, it is ground by adding water by a ratio of 1:8 and boiled using medium heat. It is then filtered with gauze.

2.4. Soyghurt production
The ingredients are soy milk, 5% sugar and skim (0%, 5%). They are stirred until dissolved and pasteurized at 80-90°C for 30 minutes. Then, they are cooled to a temperature of 45°C and added a 2% starter, incubated at 37°C for 10, 12 and 14 hours. The sample is put in a refrigerator at 5°C to stop the fermentation process [6].

2.5. Measurement of LAB amount and pH
The amount of lactic acid bacteria in soyghurt is calculated by culture in Mann Rogossa and Sharp (MRS) media based on the Total Plate Count (TPC) method using the pour plate technique. The unit of measurement is provided in CFU/ml while the pH is measured by a pH meter.

2.6. Data analysis
The data in this study is analyzed by using Shapiro-Wilk normality test (small sample size), independent sample t test if the data are normally distributed, or Mann Whitney test if the data are not normally distributed. Besides, the study also uses One Way ANOVA test (F test) to compare more than 2 groups of samples when the data are normally distributed. Otherwise, the Kruskal Wallis test is used, or Multiple Comparison test, if H₀ is rejected in the ANOVA One Way test is, using LSD (Least Significant Different). All of the calculations are performed by using SPSS for Windows 23 software.
3. Results and discussion

3.1. Lactid acid bacteria

The number of lactic acid bacteria in soyghurt is the indicator of microbiological quality [7]. Referring to the SNI standard 2009, the number of lactic acid bacteria in yoghurt is $10^7$/ml. The result shows that the addition of skim and fermentation time affects the growth of lactic acid bacteria. Additional 5% skim can increase the number of lactic acid bacteria (Figure 1). The average of lactic acid bacteria in soyghurt with additional 5% skim (average: $1.76 \times 10^8$ CFU/ml) is higher than the skim soyghurt without skim (average: $5.9 \times 10^6$ CFU/ml).

The number of lactic acid bacteria also increases along with the length of fermentation time, but there is no significant difference ($p>0.05$) on the average number of soyghurt lactic acid bacteria in the fermentation duration of 10, 12, and 14 hours in soyghurt without skim or by additional 5% skim. The highest amount of lactic acid bacteria is obtained from soyghurt with extra 5% skim with 14-hour fermentation time ($2.2 \times 10^8$ CFU/ml) as shown in Table 1.

| Fermentation Duration | 0 % P-value | 5 % P % | P-value |
|------------------------|-------------|---------|---------|
| 10 hours               | $2.0 \times 10^6 \pm 0.52$ | 0.228   | $1.28 \times 10^8 \pm 1.058$ | 0.108   |
| 12 hours               | $5.9 \times 10^6 \pm 0.66$ | 1.8 x $10^8 \pm 3.143$ |         |
| 14 hours               | $9.8 \times 10^6 \pm 0.17$ | 2.2 x $10^8 \pm 6.798$ |         |

Figure 1 shows an increase in the number of lactic acid bacteria in soyghurt. The increase is perceived to be caused by the addition of nutrients and sufficient time for lactic acid bacteria to grow. The 14-hour fermentation is a rapid breeding phase of the growth of lactic acid bacteria in this fermentation process, where the bacterial propagation takes place the soonest. The other factor which influences the growth and survival of lactic acid bacteria is chemical composition and nutrient content [8]. Additional 5% skim will increase protein in the media, so the growth of lactic acid bacteria is able to break down proteins, and the peptides will increase. Besides, an additional 5% skim will provide lactose. Lactose is a carbohydrate in skim and is a substrate which can be fermented by lactic acid bacteria [9].

![Figure 1](image-url). The comparison of the amount of soyghurt lactic acid bacteria
3.2. pH

pH is closely related to the acid content produced with fermentation. The increase in the level of lactic acid in fermentation is always in line with the decrease in the pH of fermented milk [7]. The average pH of soyghurt with additional 5% skim (mean: 3.4) is lower than that of soy without skim (mean: 3.63). There is a significant pH difference (p<0.05) in the 10, 12, and 14-hour fermentation treatment. The average pH of soybean without skim with 10-hour fermentation (3.623 ± 0.15a) is significantly different from that in 12 hours (3.663 ± 0.15b), and 14 hours (3.727 ± 0.021c). Likewise, the average pH of soyghurt with additional 5% in 10-hour fermentation treatment (3.56 ± 0.06a) is significantly different from that in 12 hours (3.39 ± 0.06b) and 14 hours (3.25 ± 0.06c) as shown in Table 2.

Figure 2 provides the description of the decrease in pH of soyghurt. The additional 5% skim and the longer the fermentation indicates the smaller soyghurt pH. The addition of substrate and fermentation time leads to the increase of microbial activity and the number of lactic acid bacteria, so more and more extracts are fermented. Lactobacillus Bulgaricus in soyghurt can stimulate Streptococcus Thermophilus. The rapid growth of Streptococcus Thermophilus will produce lactic acid, which causes a decrease in pH [9-11].

| Fermentation Duration | Skim 0 %  | Skim 5 %  | P-value |
|-----------------------|----------|----------|---------|
| 10 hours              | 3.73 ± 0.15a | 3.56 ± 0.06a | 0.001   |
| 12 hours              | 3.66 ± 0.15b | 3.39 ± 0.06b | 0.003   |
| 14 hours              | 3.62 ± 0.02c | 3.26 ± 0.06c | 0.003   |

**Note:** The different lowercase superscripts in the same column prove significant differences (p <0.005)

Fermentation process gave significant effect on the nutritional value of soy milk. Soyghurt has several effects caused by lactic acid bacteria in the fermentation process, which is to balance the digestive system, reduce cholesterol levels, prevent cancer, and overcome fungal and bacterial infections. Some studies have found that improving the quantity and type of protein can reduce the risk of kidney failure. Limiting protein in the diet reduce kidney injury while a high intake of protein will accelerate the development of chronic kidney disease [12]. In recent years, researchers have assumed that altering the dietary habits of animal protein with vegetable protein, especially protein in soybeans can be considered as functional food for suppressing chronic kidney disease.
Soybean is a high source of protein and contains important isoflavones such as genistein and daidzein. Soy protein contains a unique amino acid profile and is different from animal peptides. Amino acids in soy have a significant effect on high blood pressure and hyperlipidemia. Therefore, soy protein affect kidney function [13]. Azadbakht and Esmaillzadeh [14] prove that consumption of 0.8 g/kg of soy protein for 7 weeks can reduce urine urea nitrogen, proteinuria, and blood sodium but calcium and potassium levels do not change.

The results of this study showed that the optimum conditions for the production of the soyghurt gave products that have a high number of lactic acid bacteria by addition of 5% of skimmed milk and 14 hours of fermentation. Other study by Syamsuddin et al. [15] is in line with this research which proved that increasing skimmed milk concentration lead to increase in the number of lactic acid bacteria and decrease pH and syneresis. The study also proved that addition of 15% skimmed-milk and 5% starter was successfully produced the highest of lactic acid product. The effect of starter concentration on soyghurt also influence on the nutrition value [15]. According to Indonesian standard (SNI 01-2981-2009), the minimum amount of lactic acid bacteria for soyghurt production is $10^7$ CFU/ml. Thus, this study proved that the production of soyghurt met with Indonesia standard because the amount of lactic acid bacteria reaches to $2.2 \times 10^8$ CFU/ml.

4. Conclusion
The addition of 5% skims and fermentation time affect the number of lactic acid bacteria and pH in soyghurt. The current research concludes that the addition of 5% skims and 14-hour fermentation results in soyghurt with the highest amount of lactic acid bacteria ($2.2 \times 10^8$ CFU / ml) with pH 3.25.

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