The Effect of Different Starter Cultures and Dextrose on Viability of Lactic Acid Bacteria and pH of Fermented Milk At 43 °C

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Abstract. The purpose of this research was to study the effect of different starter cultures and the addition of dextrose on viability of LAB and pH value of fermented milk. Streptococcus thermophilus and Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus plantarum IS-10506 were used as mixed starter cultures, incubated at 43°C for 8 hours, with or without the addition of dextrose. The total viable counts of LAB and pH value were assessed. Each combination of starter cultures showed significant increased on the viable counts of LAB, and decreased the pH value of fermented milk during 8 hours incubation. Addition of 2% dextrose tended to increase viable counts of LAB by 165.8 % of milk fermented with mixed starter cultures of S. thermophilus and L. plantarum IS-10506, extending log phase from 6 to 8 hours, while without 2 % dextrose tended to decreased by 40 %. No significant effect of addition of 2% dextrose on viable counts of yogurt starters in yogurt. Mixed starter cultures of S. thermophilus and L. plantarum IS-10506 is potential starter cultures of fermented milk with addition of 2 % dextrose at 43°C for 8 hours incubation time to produce probiotic based functional drink.

Keywords: dextrose, fermented milk, mixed cultures, pH value, viable counts of LAB

1. Introduction
Fermented milk is known as one of dairy products with higher nutrient content and a longer shelf-life than milk, involving lactic acid bacteria as good bacteria. One type of fermented milk product is Yogurt. According to the Codex Alimentarius (1975) [5], yogurt is a fermented milk product obtained by involving Lactobacillus bulgaricus and Streptococcus thermophilus. Fermented milk would become more functional with the addition or substituting one of the two starter cultures with probiotic. Probiotics are living microorganism, which when administered in adequate amounts confer a health benefit on the host [7]. To provide positive effects on the host, the number of viable counts of the probiotics should be consumed in adequate amount, and according to Abe (2010) [1], 10⁶ CFU/day, and bacteria used in probiotic food products mostly belong to Lactobacillus and Bifidobacterium. Lactobacillus plantarum IS-10506 is a strain of lactic acid bacteria isolated from Dadih [13]. It is a potential novel probiotic due to the ability of adhesion and colonize well in digestive tract [6].
study aimed to determine the effect of different combination of starter cultures and addition of dextrose on the viability of LAB and the pH value of fermented milk.

2. Materials and Method

2.1. Sources and maintenance of cultures

Starter cultures consist of yogurt cultures (L. bulgaricus and S. thermophilus) obtained from DUPONT, and mixed cultures of S. thermophilus and L. plantarum IS-10506. Each of the pure culture of S. thermophilus (0.9x10^7 CFU ml^-1) and L. plantarum (1.54x10^7 CFU ml^-1) was inoculated at 1% v/v, as well as 2 g yogurt culture powder (5.8x10^8 CFU) was inoculated into each of 100 ml, 100 ml and 200 ml, respectively, of skim milk liquid medium and mixed well, as mother cultures and stored in a chiller (5°C) for 24 hours, for further use in manufacturing of fermented milk.

2.2. Preparation of fermented milk

Skim milk powder (10%) dissolved in 1000 ml of water and pasteurized in autoclave at 105°C for 2 minutes. Then, 2% dextrose (v/v) was added to skim milk liquid medium, or without 2% dextrose. Mother culture of yogurt starter at 4% (v/v), (2.32x10^7 CFU ml^-1) was inoculated into the 1000 ml pasteurized skim milk liquid medium and incubated at 43°C for 8 hours. Meanwhile, 2% (v/v) mother culture of L. plantarum IS-10506 at (7.7x10^8 CFU ml^-1) was inoculated first into the skim milk liquid medium, incubated for 4 hours at 43°C, followed by the addition of 2% (v/v) mother culture of S. thermophilus (4.5x10^8 CFU/ml^-1) to the milk precultured with L. plantarum IS-10506, and incubated for 8 hours. Fermented milk was prepared with different mixed starter cultures, with or without 2% dextrose. The experiments consisted of one variable (Different starter cultures) with four levels of treatment carried out with two replication and 2 times of observation:
- Culture S. thermophilus & L. plantarum IS-10506 (without dextrose)
- Culture S. thermophilus & L. plantarum IS-10506 (with dextrose)
- Culture S. thermophilus & L. bulgaricus (without dextrose)
- Culture S. thermophilus & L. bulgaricus (with dextrose)

2.3. Microbiological analysis

2.3.1 Viable counts of lactic acid bacteria (LAB)

Total viable counts of LAB was assessed by using plate count method on de Man Rogosa and Sharpe (MRS) agar medium. Sampling was conducted once every 2 hours with a serial dilution of the 10^{-1} to 10^{-9}. Furthermore, 1 ml sample was poured on MRS agar then incubated at 37 °C for 24 hours.

2.3.2 LAB Morphological analysis

Morphology of lactic acid bacteria was observed by Gram staining to confirm the type and shape of each cell. Dried reparation prepared, and then applied gram dye primary strain, crystal violet, iodine, alcohol, and counter stained with safranin. After that, the morphology of the bacteria was observed using a microscope with a magnification of 1000×.

2.3.3 Catalase test

The test was performed according to the BSN (2009) method. One ose colonies of bacteria was taken and placed on a glass object. Then a solution of 3% H_2O_2 dropped over the preparation. Positive result is shown by the presence of air bubbles on the object.

2.4. Measurement of pH value

Measurement of pH value was carried out by using pH meter (Lab 860, SI Analytics, Xylem Global Brads, New York, US).

2.5. Statistical analysis

Data analysis method used was one-way ANOVA followed by Duncan test.
3. Results and Discussion

3.1. The effect of dextrose on the LAB viable counts in fermented milk

Figure 1 and Figure 2 show the viable counts of lactic acid bacteria in milk fermented with a combination of S. thermophilus and L. plantarum IS-10506 culture, and yogurt starter cultures, respectively, with and without the addition of dextrose.

![Figure 1](image1.png)  
**Figure 1.** Viable counts of LAB of fermented milk with S. thermophilus and L. plantarum IS-10506 at 43°C

![Figure 2](image2.png)  
**Figure 2.** Viable counts of LAB of fermented milk with S. thermophilus and L. bulgaricus at 43°C

Figure 1 shows an increase of viable counts of LAB in milk fermented with combination of S. thermophilus and L. plantarum IS-10506 cultures during 6 hours incubation with and without the addition of dextrose, the viable counts were in a range of 7.98 log CFU ml^{-1} (9.50 x 10^{7} CFU ml^{-1}) to 9.94 log CFU ml^{-1} (8.75 x 10^{8} CFU ml^{-1}) (0-6 hours), and tended to decreased after 2 more hours incubation (at 8 hours), from 9.94 log CFU ml^{-1} (8.75 x 10^{8} CFU ml^{-1}) into 9.53 log CFU ml^{-1} (5.26 x 10^{8} CFU ml^{-1}), the viability was decreased by 40 % without dextrose. In the presence of dextrose, the viable counts of LAB was in a range of 8.03 log CFU ml^{-1} (1.08 x 10^{7} CFU ml^{-1}) to 9.91 log CFU ml^{-1} (8.87 x 10^{8} CFU ml^{-1}) during 8 hours incubation time, and after 2 more hours incubation at 43°C, the viability increased by 165.8 %, from (5.35 x 10^{8} to 8.87 x 10^{9}) CFU ml^{-1}.

Longer incubation time significantly increased the viable counts of LAB in milk fermented with S. thermophilus and L. plantarum IS-10506. The addition of dextrose did not increase or decrease the viable counts of LAB in yogurt, after 8 hours incubation, to increase the viable counts of LAB in milk fermented with combination of S. thermophilus and L. plantarum IS-10506 from 6 to 8 hours incubation, whereas the cultures without dextrose tended to decrease the viable counts at 6 to 8 hours. The log phase was extended up to 8 hours with the presence of 2 % dextrose. According to Zahroh et al., (2011) [15], dextrose serves as the necessary nutrients for bacterial growth.

Milk fermented with yogurt starter cultures, S. thermophilus and L. bulgaricus with and without the addition of dextrose showed comparable growth of lactic acid bacteria during 8 hours (Figure 2), while without dextrose constantly increasing from 0 to 8 hours of incubation time, ranging between 7.77 log CFU ml^{-1} (1.12 x 10^{8} CFU ml^{-1}) to 10.07 log CFU ml^{-1} (1.27 x 10^{9} CFU ml^{-1}), increased by 11,339 %. Meanwhile, addition of dextrose increased from 0 to 6 hours, from 7.20 log CFU ml^{-1} (1.63 x 10^{7} CFU ml^{-1}) to 9.98 log CFU ml^{-1} (9.72 x 10^{9} CFU ml^{-1}), increased by 596.43%, and began to decrease at 8 hours to be 9.90 log CFU ml^{-1} (8.05 x 10^{9} CFU ml^{-1}), by 17.2 %.

Addition of dextrose significantly extend log phase to 8 hours incubation time of mixed cultures combination of S. thermophilus and L. plantarum IS-10506. Since L. plantarum IS-10506 is mesophilic bacteria, the presence of dextrose supported its growth under unfavourable condition. Yogurt starter grew well at their optimum growth temperature of 43°C. Addition of dextrose seems to speed up the growth rate, hence, at 6 hours incubation, it has come to optimum growth or end of log...
phase, and come into stationary phase the next 2 hours incubation. Since 43°C is an optimum growth temperature for thermophilic group, the yogurt starters, S. thermophilus and L. bulgaricus [13] resulted in higher viable counts after 8 hours incubation. However, both starter cultures, yogurt cultures without dextrose and mixed starter of S. thermophilus and L. plantarum IS-10506 with the addition of dextrose, showed good growth in skim milk as shown by their growth rate during 8 hours incubation which is in the log phase, and had comparable ability as starter cultures.

3.2. The effect of dextrose on pH value
Addition of dextrose showed no significant effect on pH value of yogurt as well as fermented milk (Figures 3 and 4). Mixed cultures of S. thermophilus and L. plantarum IS-10506 at 43°C, with or without dextrose started to lower the pH value at 4 hours incubation, and the longer the incubation time, the lower the pH during 8 hours incubation. This phenomenon was in line with the growth pattern of the LAB, at the first two hours it was in lag phase even in the presence of dextrose (Figure 1). On the other hand, the pH of yogurt was lower at 2 hours incubation (Figure 4), in line with the growth pattern of LAB as shown in Figure 2, viable counts of LAB started to increase at week 2, producing lactic acid bacteria which is responsible in lowering the pH of yogurt. Figure 4 also confirmed that with and without dextrose, the pH value of yogurt were comparable, means, dextrose is not required to be added in yogurt fermentation.

3.3. The correlation of LAB viability and pH value
The correlation between the viable counts of lactic acid bacteria with pH value of fermented milk showed by R² (Figures 5-8).
The R\(^2\) values were in a range of 0.7876 to 0.9658 (Figures 5, 6, 7, and 8), means viability of lactic acid bacteria in a range of 78.76% to 96.58%, responsible for lowering the pH value. While about 3.42 to 21.24% is influenced by other factors. The variable x (LAB) gives a strong influence on the variable y (pH value) because the value of R\(^2\) generated close to a value of 1, or it can be said the viable counts of lactic acid bacteria are negatively correlated (inversely) against pH value, so that the results are strongly correlated.

The addition of dextrose in manufacturing of fermented milk supported the growth of lactic acid bacteria, especially \(L.\) \textit{plantarum IS-10506}. According to Surono \textit{et al} (2008) [12], glucose supplies energy to microbial cells at stationary phase. \(L.\) \textit{plantarum IS-10506} continued to consume glucose and produce ATP for cell maintenance and survival. The addition of dextrose supported the growth of lactic acid bacteria involved as mixed starter cultures, as shown by an increase of lactic acid bacteria viable counts, producing more lactic acid bacteria, hence, lowering the pH of fermented milk.
3.4. The confirmation of lactic acid bacteria

Table 1 shows Gram staining and catalase test of each bacterial culture. *L. plantarum* is a Gram-positive lactic acid bacteria as shown by a purple Gram staining result, catalase negative characterized by the absence of gas bubbles generated, and has a rod-shaped cells, form pairs or chains. *S. thermophilus* culture is a Gram-positive bacteria, catalase negative and has a cocci cells, and tended to form chain. Meanwhile, *L. bulgaricus* is a Gram-positive bacteria, catalase negative, and rod-shaped. Based on Bergey’s Manual of Systemic Bacteriology (1974), *Lactobacillus* is catalase-negative, rod-shaped, and gram positive. Hence, the purity was confirmed. Morphologies of the microorganisms employed in this study is shown in Figures 9 to 11.

| Bacteria          | Gram staining | Catalase test | Morphology  |
|-------------------|---------------|---------------|-------------|
| *L. plantarum*    | +             | -             | Rod-shaped  |
| *L. bulgaricus*   | +             | -             | Rod-shaped  |
| *S. thermophilus* | +             | -             | Cocci       |

Figure 9. Morphology of *S. thermophilus*

Figure 10. Morphology of *L. plantarum* IS-10506

Figure 11. Morphology of *L. bulgaricus*

4. Conclusion

Milk fermented with yogurt cultures, *S. thermophilus* and *L. bulgaricus* showed a higher viable counts and lower pH value than the milk fermented with mixed cultures of *S. thermophilus* and *L. plantarum* IS-10506 at 43°C for 8 hours. The addition of 2% dextrose in fermentation of milk at 43°C supported the growth of *L. plantarum* as mixed culture with *S. thermophilus*, and it is recommended to add 2% dextrose to ferment milk with *L. plantarum* and *S. thermophilus*, to utilize probiotic as starter culture,
and producing functional drink. However, dextrose is not recommended to be added in yogurt fermentation at 43°C.

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