Physio-chemical, Microbiology, and Preference of Probiotic Fresh Soft Cheese Using *Lactobacillus plantarum* IS-10506 and *Streptococcus thermophilus* as Mixed Starter Culture

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**Abstract.** Probiotics are live microorganisms with various beneficial effect on human health when consumed in adequate amount. Soft cheese is one of fermented milk products involving lactic acid bacteria (LAB) which may be potential as functional food. Concentration ratios of starter culture *Lactobacillus plantarum* IS-10506 and *Streptococcus thermophilus* were 1:1, 2:1, and 1:2 (v/v) at 5%, 10%, and 15% (v/v) at $10^6$ CFU mL$^{-1}$ cultures and incubated at 37 °C for 90 minutes. Chemical (pH and moisture content), microbiology (LAB total count), and sensory (preference test) were analyzed. The results showed that there was no significant difference ($p$-value < 0.05) on the viability of LAB (in a range of $6 \times 10^7$ to $1.71 \times 10^8$ CFU mL$^{-1}$) and on cheese yield (224-268.84 g L$^{-1}$). Cheese fermented with 1:1 (v/v) *Lactobacillus plantarum* IS-10506 and *Streptococcus thermophilus* of 10% cultures (v/v) was the most preferred, scored 3.75 out of 5 in hedonic test for overall acceptability and the viable LAB was $1.71 \times 10^8$ CFU mL$^{-1}$ with pH 4.95. The total yield was 257.54 g L$^{-1}$ and its moisture content was 66.45%.

**Keywords**: probiotic fresh soft cheese, starter cultures, *Lactobacillus plantarum* IS-10506, *Streptococcus thermophilus*

1. **Introduction**

Healthy lifestyle trends with more health concern are growing as shown by healthy food and functional foods in the market [1]. As a consequence, an increasing number of food producers are beginning to produce functional foods, described as food with additional health benefits in addition to its existing nutrient content [2], one of which is fermented food.

Fermentation is a way of processing food either with addition of starter culture spontaneous one, involving microbes naturally present in raw materials. Fermentation is an ancient technique to preserve food. The microbes involved will convert food components resulting in desirable sensory attributes in foods [3]. In addition to that, lactic acid bacteria involved in the fermentation may be a potential probiotic World Health Organization defined probiotics as live microorganisms that serve various beneficial effects on human health when consumed in adequate amount [4]. The probiotic function is strain-specific and dose dependent. Some studies have been conducted and showed the minimum effective dosage for probiotics consumption is $10^6-10^7$ CFU mL$^{-1}$ [5].
LAB is Generally Recognized as Safe (GRAS) bacteria, which is safe to consume and non-toxic to human [6-7]. Lactobacillus plantarum IS-10506 is a novel probiotic isolated from dadih, traditional fermented buffalo milk popular among people in West Sumatera, Indonesia [8]. Several studies reported L. plantarum IS-10506 has potential probiotics characteristics (such as gastric and bile acid resistance, adherence to mucosal surface or gastrointestinal cell lines, and competition against pathogenic bacteria) better than other L. plantarum strains [8-12].

Dairy products such as cheese are being fermented. During cheese manufacture, curd and whey were separated due to milk coagulation. There are several types of cheese, namely soft cheese, semi-soft cheese, and hard cheese. Soft cheese can be consumed directly after fermentation process because unlike the other two, it does not undergo aging or maturation process. In addition, due to the short manufacturing process, probiotic bacteria in soft cheese can survive as viable probiotics. The objectives of this study were to evaluate the best L. plantarum IS-10506 and S. thermophilus concentration as mixed starter cultures in producing soft cheese, and to evaluate the physio-chemical, microbiological, and sensory characteristics of the manufactured soft cheese.

2. Materials and Method
This study was conducted in three main stages. In the first stage, each bacteria standard and growth curve were taken using HiMedia de Mann Rogosa and Sharpe Broth (MRS) broth medium to prepare mother culture. In the second and third stage, mix cultures growth pattern and cheese pH were assessed at different L. plantarum IS-10506 and S. thermophilus concentrations, also different starter culture concentration.

2.1. Standard Curve
Standard curve was used to determine the initial number of LAB in MRSB to prepare mother culture. One oose of LAB was taken from HiMedia MRS agar and put into 10 mL of MRS broth incubated at 37 °C for 24 hours, then diluted to obtain optical density (OD) of 0.2-0.8. The viable counts of LAB cells were enumerated for each OD value by spread plate. The OD range was used to measure the viable count expected was in log phase. The standard curve equation was then obtained using a graph where the X axis is OD values and the Y axis the amount of cells.

2.2 Growth Curve
Growth curve was done to determine the age of culture used. One oose of lactic acid bacteria (LAB) was taken from MRS agar and put into 10 mL of MRS broth, at 37°C incubated for 24 hours. The initial viable count used was 10^6 CFU mL⁻¹, which was equivalent to OD 0.14 for L. plantarum IS-10506 and 0.1 for S. thermophilus, obtained from standard curve. The OD value of the culture was measured every two hours for 24 hours.

2.3 Mother Culture Preparation
Mother culture was made for each LAB. After LAB activation and cultivation, the culture was diluted so that the OD value from standard curve was obtained. The culture is then incubated at 37°C for 24 hours, harvested by centrifugation (1643 G, -4°C, 15 mins). Cell pellets were collected and dissolved in 50 mL of 10% OpenCountry (New Zealand) whole milk product (WMP).

2.4. Soft Cheese Making
Ten percent of L. plantarum IS-10506 and S. thermophilus culture (10^6 CFU mL⁻¹) was added to pasteurized WMP at concentration ratios of 1:1, 1:2, and 2:1 (v/v), then pure liquid calf rennet (obtained from Dunia Keju), lactic acid, and 2% dextrose were added, and incubated at 37°C for 90 minutes. When incubation was completed, whey was drained. Then, 1% (w/w) NaCl solution was added to the curd. Figure 2 shows the incubation process and separation of whey. After obtaining the best concentration ratio for Lactobacillus plantarum IS-10506 and Streptococcus thermophilus, soft cheese was made by adding 5%, 10%, and 15% mixed starter cultures.
2.5. Physical analyses of soft cheese
The total yield produced was obtained from curd weight. Curd was weighed using digital scale.

2.6. Chemical analyses of soft cheese
Cheese pH was measured every 30 minutes using pH meter during incubation period. Moisture content was analyzed by heating ±5 grams of cheese sample in an oven at 105°C for 4 hours [13]. Cheese moisture content was calculated as follows:

\[
\text{Moisture content} = \frac{A - B}{B} \times 100\%
\]

A = dish weight + sample weight before heating
B = dish weight + sample weight after heating

2.7. Microbiological analyses
The total viable counts of lactic acid bacteria was determined by pour plating of serial dilution (\(10^{-5}, 10^{-6}, 10^{-7}\)) by using HiMedia Lactobacillus MRS Agar and incubated at 37 °C for 48 hours [14]. LAB total viable counts was measured every 30 minutes during incubation process. Yeast and moulds were enumerated by using HiMedia Potato Dextrose Agar (PDA) [15]. Coliform bacterial count was determined using HiMedia Violet Red Bile Agar plate (VRBA). Total plate count was conducted to see if there is any other bacterial contaminants aside from LAB by pour plating using HiMedia Plate Count Agar.

2.8. Sensory evaluation of soft cheese
Soft cheese was subjected to sensory evaluation using 20 panelists. 20 panelists was used as a minimum number of panelists for laboratory sensory evaluation testing and to achieve significant results [16-17]. Each panelist was asked to rate cheese samples’ on texture, taste, flavor, and overall acceptability as shown in Table 1 [18].

| Hedonic Scale | Texture          | Taste (Saltyness) | Flavor       | Overall acceptability |
|---------------|------------------|-------------------|--------------|-----------------------|
| 1             | Soft             | Bitter            | Off flavor   | Dislike               |
| 2             | Slightly soft    | Slightly salty    | Slightly strong | Fair                  |
| 3             | Hard             | Salty             | Strong       | Good                  |
| 4             | Very hard        | Very salty        | Very strong  | Very good             |
| 5             | Extremely hard   | Extremely salty   | Extremely strong | Excellent             |

2.9. Statistical analyses
All data obtained were analyzed using ANOVA One-way to determine whether there was any significant difference between physio-chemical and microbiological properties for each soft cheese (p-value <0.05).
3. Results and Discussion

3.1. Standard and growth curves of Lactic Acid Bacteria

The initial number of LAB cells was set at $10^6$ CFU mL$^{-1}$ as shown in Figures 2 and 3. OD value for *L. plantarum* IS-10506 and *S. thermophilus* obtained were 0.14 and 0.10 repetitively with starting number of $10^6$ CFU mL$^{-1}$.

Figure 2. *Lactobacillus plantarum* IS-10506 standard curve

Figure 3. *Streptococcus thermophilus* standard curve

Figure 4 shows each of growth curves. Three growth phases for *S. thermophilus* were observed at the growth curve, which were lag phase at 0 to 2 hours, log phase from 2 to 6 hours, and entering stationary phase at 8 hours. Meanwhile, only two growth phases were obtained for *L. plantarum* IS-10506, which were log phase from 0 to 10 hours, and entering stationary phase at 10 hours. Doubling time for *L. plantarum* was 68 minutes, while *S. thermophilus* was 45 minutes. The different phases obtained was due to the incubation temperature at 37 °C, which is optimum temperature for *L. plantarum*, so *L. plantarum* quickly adapted to the medium as compared to *S. thermophilus* which the optimum growth temperature at higher temperature, 45°C [19-20]. However, the pH obtained for *L. plantarum* as well as *S. thermophilus* at logarithmic growth was not low enough to coagulate protein in cheese making, so an overnight (24 hours) culture was used. Each of the LB were still in stationary phase at 24 hours incubation, which means the bacteria still have its ability to grow when introduced to new growth medium [21].

Figure 4. *L. plantarum* IS-10506 and *Streptococcus thermophilus* growth curve
3.2. Soft cheese with different *L. plantarum* IS-10506 and *S. thermophilus* concentration ratio

*Lactobacillus plantarum* IS-10506 and *S. thermophilus* mix cultures growth pattern and changes in cheese pH during incubation for soft cheese A (*L. plantarum* IS-10506 : *S. thermophilus* = 1:1), B (*L. plantarum* IS-10506 : *S. thermophilus* = 2:1), and C (*L. plantarum* IS-10506 : *S. thermophilus* = 1:2) can be seen in Figure 5.

![Figure 5](image-url)

**Figure 5.** Mixed cultures growth pattern and pH changes of soft cheese A (a), B (b), and C (c)

Mixed cultures A showed a rapid growth rate as shown by highest cell numbers at 60 minutes at 37 °C as well as final cell numbers at 90 minutes, 37 °C. This is due to the protocooperation between *Lactobacillus* genus bacteria and *S. thermophilus*. *Lactobacillus* will provide essential amino acid and peptide for *S. thermophilus* growth, while *S. thermophilus* will provide folic acid for *Lactobacillus* [7], so that balanced concentration of each species results in better and more stable bacterial growth as shown in Table 2. Soft cheese with *L. plantarum* IS-10506 and *S. thermophilus* concentration ratio of 1:1 had the highest number of LAB cells, which was 1.22×10⁶ CFU mL⁻¹.

| Table 2. Growth rate pattern of LAB in soft cheese with different *L. plantarum* IS-10506 and *S. thermophilus* concentration ratios |
|---------------------------------------------------------------|
| Increasing growth rate (log CFU mL⁻¹ min⁻¹) | Highest cell number (10⁶ CFU mL⁻¹) | Decreasing growth rate (log CFU mL⁻¹ min⁻¹) | Final cell number (10⁶ CFU mL⁻¹) |
| A | 0.010 | 236 | 0.01 | 122 |
| B | 0.001 | 190 | 0.01 | 78 |
| C | 0.030 | 174 | 0.01 | 60 |

The soft cheese fermented with mixed cultures A showed the lowest pH as compared to mixed cultures B and C (Figures 5b and 5c). *Lactobacillus* and *S. thermophilus* have a proto-cooperation...
interaction in producing lactic acid [7]. Involving both species as starter cultures with balanced concentration will increase the production of lactic acid resulted in lower pH. Initial pH of milk was 6.36 ± 0.01 and gradually decreased until 30 minutes incubation and there is an increased in pH until 90 minutes incubation due to the production of glycomacropeptide. Lactic acid and chymosin in rennet act as coagulant, which will break down κ-casein, protein in milk, into para κ-casein and glycomacropeptide [22]. Glycomacropeptide has a base chain that will act as buffer in a solution resulting in increased pH [23]. Table 3 shows physical, chemical, microbiology, and sensory parameter of cheese A, B, and C, and compared with standard.

Table 3. Physical, chemical, microbiology, and sensory parameter for cheese A, B, and C

| Parameter     | A                | B                | C                | Standard   |
|---------------|------------------|------------------|------------------|------------|
| LAB (CFU mL⁻¹) | (1.2±0.08)×10⁸  | (7.8±0.16)×10⁷  | (6±0.04)×10⁷     | 10⁶-10⁷    |
| pH            | 4.89±0.14        | 5.09±0.21        | 5.2±0.2          |            |
| Moisture content (%) | 66.4±1.6        | 66.51±5.49       | 70.03±1.33       | >65% [24]  |
| Yield (g L⁻¹) | 252±12           | 245.5±9.5        | 224±6            | -          |
| Overall acceptability | #1             | #3              | #2              | -          |
| PCA           | <10⁴             | <10⁴             | <10⁴             | Max.10² [25]|
| PDA           | Negative         | Negative         | Negative         | -          |
| Coliform      | <10¹             | <10¹             | <10¹             | Max. 10² [25]|

Table 4. Mean scores for preference of cheese A, B, and C

| Soft cheese | Preferences |
|-------------|-------------|
|              | Texture | Taste | Flavor | Overall Acceptability |
| A            | 2.3     | 1.7   | 2.7    | 3.2                   |
| B            | 2.2     | 2.4   | 1.8    | 2.5                   |
| C            | 1.7     | 2.3   | 1.3    | 2.95                  |

As shown in Table 4, soft cheese A tended to have higher preference compared with soft cheese B and C. Soft cheese C with higher S. thermophilus concentration produced cheese with softer texture. S. thermophilus is dominant in producing diacetyl, a volatile compound renowned for its buttery characteristic [26-27], while soft cheese with higher L. plantarum IS-10506 concentration produced more granulated cheese as a product of casein coagulation. Soft cheese with L. plantarum IS-10506 and S. thermophilus concentration ratio of 1:1 had a combined better characteristic.

Figure 6. Soft cheese with L. plantarum IS-10506 and S. thermophilus different concentration ratio (left = 1:1, middle = 2:1, right = 1:2)

The best L. plantarum IS-10506 and S. thermophilus concentration ratio (A) as selected for cheese making with different concentrations.

3.3. Effect of different starter culture concentration on viable counts of LAB and pH value of soft cheese

L. plantarum IS-10506 and S. thermophilus mix cultures growth pattern and changes in pH during incubation for soft cheese with 5%, 10%, and 15% cultures (v/v) are shown in Figure 7.
Figure 7. Growth pattern of mix cultures and changes in pH of soft cheese with 5% (a), 10% (b), and 15% (c) culture.

Soft cheese with 15% cultures had the most rapid LAB growth pattern at the starting point of incubation and reached its peak at 60 minutes of incubation time, and come to death phase as well due to limited nutrient available. On the other hand, soft cheese with 5% cultures had the lowest bacterial growth pattern compared to two other cheeses due to the fewer addition of starter cultures. Soft cheese with 10% starter cultures had a more stable LAB growth pattern and the highest cell number at the end of incubation time, which was $1.71 \times 10^8$ CFU mL$^{-1}$. There will be interactions between microbes in fermentation medium, whether it’s positive or negative interaction, thus there will be competition between individuals to gain sufficient nutrition for their growth. Too many cells will cause lack of nutrients in the medium for cell growth, but too few cell number will also cause a low growth rate. Therefore, the nutrients in the medium must be adequate to the number of microbes to support optimal microbial growth [28]. Table 5 shows growth rate of the mixed cultures at different concentrations.

Table 6 shows physical, chemical, microbiology, and preference of each cheese with 5%, 10%, and 15% starter cultures. It could be observed from Table 6 that the highest viable counts of LAB obtained from soft cheese with 10% cultures. Table 7 shows preference on soft cheese with different starter culture concentration. As shown in table 7, soft cheese with 10% culture tended to have higher preference compared with soft cheese with 5% and 10% culture. Figure 8 shows soft cheeses produced with 5%, 10%, and 15% culture.
Table 5. Increasing and decreasing LAB cell growth rate of soft cheese with different concentration of starter culture

|                | Increasing growth rate (log CFU mL\(^{-1}\) min\(^{-1}\)) | Highest cell number (10\(^6\) CFU mL\(^{-1}\)) | Decreasing growth rate (log CFU mL\(^{-1}\) min\(^{-1}\)) | Final cell number (10\(^6\) CFU mL\(^{-1}\)) |
|----------------|----------------------------------------------------------|-----------------------------------------------|--------------------------------------------------------|-----------------------------------------------|
| 5%             | 0.01                                                     | 219                                           | 0.01                                                   | 122                                           |
| 10%            | 0.01                                                     | 236                                           | 0.01                                                   | 171                                           |
| 15%            | 0.01                                                     | 283                                           | 0.01                                                   | 126                                           |

Table 6. Physical, chemical, microbiology, and preference test of cheese with 5%, 10%, and 15% starter cultures

| Parameter                      | 5%                              | 10%                              | 15%                              | Standard          |
|--------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------------|
| LAB (CFU mL\(^{-1}\))         | (1.22±1.8)×10\(^8\)            | (1.71±0.33)×10\(^8\)            | (1.26±0.85)×10\(^8\)            | 10\(^6\)-10\(^7\) |
| pH                            | 5±0.3                           | 4.95±0.25                       | 4.92±0.08                       | -                 |
| Moisture content (%)           | 77.73±0.05                      | 66.45±0.05                      | 72.62±0.01                      | >65% [24]         |
| Yield (g L\(^{-1}\))          | 237.12±1.96                     | 257.54±2.24                     | 268.84±8.8                      | -                 |
| Overall acceptability          | #3                              | #1                              | #2                              | -                 |
| PCA                           | <10\(^1\)                       | <10\(^1\)                       | <10\(^1\)                       | Max.10\(^2\) [25] |
| PDA                           | Negative                        | Negative                        | Negative                        | -                 |
| Coliform                      | <10\(^1\)                       | <10\(^1\)                       | <10\(^1\)                       | Max. 10\(^2\) [25] |

Figure 8. Soft cheeses with 5% culture (left), 10% (middle), dan 15% (right)

Table 7. Mean scores for preference of soft cheese with 5%, 10%, and 15% cultures

| Soft cheese | Texture | Taste | Flavor | Overall Acceptability |
|-------------|---------|-------|--------|-----------------------|
| 5%          | 1.7     | 1.6   | 1.75   | 2.25                  |
| 10%         | 2.1     | 2.3   | 2.05   | 3.75                  |
| 15%         | 2.6     | 2.3   | 2.85   | 2.5                   |

LAB confirmation test was done by Gram staining. Gram stain results for both LAB can be seen in Figure 9. Both gram stain showed gram positive bacteria. Presence of rod shape gram positive bacteria showed that soft cheese produced contained *L. plantarum* IS-10506 and coccus shape gram positive bacteria showed that soft cheese produced contained *S. thermophilus*. The results showed that the manufactured soft cheese contains probiotic.
4. Conclusion
The best *Lactobacillus plantarum* IS-10506 and *Streptococcus thermophilus* concentration ratio for soft cheese making was 1:1 of 10% culture with incubation time of 90 minutes at 37°C as shown by better preference of panelist for overall acceptability. Total yield produced was 257.54 g L⁻¹ with 66.45% moisture content and pH 4.95. Microbial analyses showed that soft cheese produced contained 1.71×10⁸ CFU mL⁻¹ of LAB.

5. Recommendation
Further research on product shelf life is needed. This study was conducted on laboratory scale, therefore it is necessary to conduct further study on larger scale for mass production.

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