Physico-chemical and microbial properties of buffalo milk yoghurt drink

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Abstract. Lack of food diversification from buffalo milk causes a lack of public interest in consuming buffalo milk even though it has good nutritional content. Therefore, it is necessary to diversify buffalo milk into yoghurt. This study aimed to make yoghurt from buffalo milk and find the effect of starter concentration and fermentation time on physico-chemical and microbial properties of buffalo milk yoghurt. This research used a completely randomized design with two factors, i.e., concentration starter (3%, 5% and 10%) and fermentation time (8 and 12 hours). Parameters analysed were physico-chemical properties, i.e. acidity, pH, soluble solids, solids non-fat, total solids, sweetness index, astringency index, and microbial properties, i.e. total lactic acid bacteria and total microbial counts. The result showed an increase in values for acidity, astringency index, total lactic acid bacteria counts, and total microbial counts in increased starter concentration and fermentation time. A reverse trend was observed for pH, soluble solids, sweetness index values in increased starter concentration and fermentation time. Total lactic acid bacteria and total microbial counts were highest in sample F (10%; 12 hours). Sample A (3%; 8 hours) has the highest total solid (15.4%) and solid non-fat (11.49%).

1. Introduction
Buffalo milk has good nutrition for the human body because its protein and fat content is quite high. It is also rich in minerals and vitamins that are important for human body [1]. Total solid, crude protein, fat, calcium, and phosphorus in buffalo milk are higher than in cow milk [2]. However, buffalo milk is rarely consumed. Therefore, food diversification should be carried out in buffalo milk to increase people’s interest to consume it. One effort to diversify buffalo milk is making of probiotic buffalo milk. Probiotics are live microbes which provide human beneficial health effect in a sufficient amounts of consumption. Probiotics in food should survive in the digestive tract and proliferate in intestines [3].

Yoghurt is fermented dairy product that contains probiotics and high in milk fat, protein, vitamin B₁₂ (cobalamin), vitamin B₆ (pyridoxine) and vitamin B₂ (riboflavin), calcium, potassium, and magnesium [4,5]. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* are generally used as probiotic for fermentation milk in making yoghurt. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have found in human feces that have consumed yoghurt, so it proves the bacteria can survive in the gastrointestinal tract [6]. Probiotics of *Bifidobacterium* and *Lactobacillus casei* can survive during 12-month refrigeration storage [2].
The consumption yoghurt can improve fasting blood glucose, prevent obesity and protect cardiometabolic disease [7,8]. Lactic acid bacteria in yoghurt can maintain gastrointestinal function. High consumption of yoghurt as based dairy product can inhibit diarrhea, infection other bacteria, colon cancer, lactose intolerance and inflammatory bowel disease [9]. Yoghurt is believed to prevent osteoporosis because it facilitates absorption of calcium [10].

Thus, the objective of this research is to yield yoghurt drink from buffalo milk and to find out the effect of starter concentration and fermentation time on physico-chemical properties (acidity, pH, soluble solid, solids non-fat, total solid, sweetness index, and astringency index) and microbial properties (total lactic acid bacteria and total microbial count).

2. Materials and methods

2.1. Materials
Fresh buffalo milk was obtained from Asam Kumbang stock farmer in Medan and freeze-dried starter culture (Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus) was also purchased from yoghurt store in North Jakarta.

2.2. Rejuvenation of bacterial starter
Rejuvenation of bacterial starter was done by inoculating 5 grams of freeze-dried starter per litre of pasteurized milk at temperature of 45°C to activate the microorganism. Freeze-dried starter culture was Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus acidophilus. Furthermore, the milk was incubated at 37°C for 8 hours until coagulation was formed to obtain a work culture. The number of lactic acid bacteria in work culture was measured as 8.35 ± 0.15 log cfu/ml.

2.3. Making of yoghurt drink from buffalo milk
Buffalo milk was pasteurized for 15 minutes with temperature of 80°C which aimed to destroy bad bacteria, and then the temperature was reduced to about 45°C. The pasteurized buffalo milk was inoculated with work culture (3%, 5% and 10%) as the first factor and incubated at 37°C with fermentation time (8 hours and 12 hours) as the second factor. Furthermore, the fermented milk or yoghurts were added boiled water (1:1) and sugar 5% to produce yoghurt drinks. These were labeled as samples A, B, C, D, E and F (Table 1). The yoghurt drinks produced were cooled rapidly to 10°C in the refrigerator for subsequent analysis.

Table 1. Yoghurts samples with starter culture and fermentation time factors

| Factors          | A   | B   | C   | D   | E   | F   |
|------------------|-----|-----|-----|-----|-----|-----|
| Starter culture  | 3%  | 5%  | 10% | 3%  | 5%  | 10% |
| Fermentation time| 8 hours | 8 hours | 8 hours | 12 hours | 12 hours | 12 hours |

2.4. Physico-chemical analysis
The acidity was the measurement by adding three drops of phenolphthalein (PP) as indicator to 10 ml yoghurt drink and mix them, then titrated against 0.1N sodium hydroxide. The total value of titrated acid calculated by converting it to the percentage of acidity [11]. The samples used standardized instrumental method to measure pH and soluble solids (Brix) [12]. The sweetness and astringency indexes were counted as the ratio of soluble solids to acidity and vice versa [13]. Total solids were calculated as the reduction of one hundred to water content. Solids non-fat were calculated as the reduction of total solids to fat. Moisture content and fat were measured by AOAC methods.
2.5. Microbiological analysis

The microbiological determination in the yoghurt drinks were measured by using Plate Count Agar (PCA) for the total plate count and incubated at 35±1°C for 48±3 hours [14] and Man Rogosa Sharpe Agar (MRSA) containing 0.02% (w/v) sodium azide for the total lactic acid bacteria and incubated at 37°C for 48 hours [15]. The colony counting range from duplicate plates was 25-250 and calculated using standard plate count formula and expressed as log colony forming unit per ml (log cfu/ml).

2.6. Statistical analysis

Data obtained from parameters analysis in two repetitions of the samples were evaluated statistically using a variance analysis (ANOVA).

3. Results and discussion

3.1. Physico-chemical properties

The physico-chemical properties of yoghurt drink from buffalo milk observed, i.e., acidity, pH, soluble solids, sweetness index, astringency index, total solids and solids non-fat are summarised in Table 2.

| Parameters                  | A               | B               | C               | D               | E               | F               |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Acidity (%)                 | 0.62±0.01       | 0.73±0.02       | 0.77±0.03       | 0.77±0.06       | 0.81±0.10       | 0.92±0.03       |
| pH                          | 4.37±0.01       | 4.24±0.03       | 4.04±0.23       | 4.07±0.11       | 3.89±0.08       | 3.85±0.07       |
| Soluble solids (%)          | 9.90±0.14       | 9.65±0.21       | 8.60±0.57       | 7.85±0.49       | 7.50±0.42       | 7.25±0.35       |
| Sweetness index             | 15.94±0.10      | 13.16±0.05      | 11.11±0.37      | 10.27±0.21      | 9.30±0.65       | 7.86±0.11       |
| Astringency index           | 0.06±0.00       | 0.08±0.00       | 0.09±0.00       | 0.10±0.00       | 0.11±0.00       | 0.13±0.00       |
| Total solids (%)            | 15.40±0.14      | 13.50±0.42      | 13.40±0.28      | 13.90±0.28      | 13.20±0.28      | 14.50±0.28      |
| Solids non-fat (%)          | 11.49±0.23      | 5.45±0.28       | 9.57±0.21       | 6.67±0.10       | 10.46±0.44      | 11.29±0.49      |

The acidity of yoghurt drink from buffalo milk ranged from 0.62 to 0.92%. The statistical analysis revealed a significant difference (p<0.05) among the yoghurt drinks samples in the acidity values observed (Table 3). The acidity values increase in increasing starter concentration and fermentation time. Sample F had a higher acidity value (0.92%) while sample A had a lower acidity value (0.62%), but 0.6% acidity is recommended for plain yoghurt [16].

Table 3. The result of analysis statistical of yoghurt drink from buffalo milk’s physico chemical parameters

| Parameters      | p-value of factor |
|-----------------|-------------------|
| Acidity         | 0.015             | 0.006             |
| pH              | 0.040             | 0.005             |
| Soluble solid   | 0.036             | 0.000             |
| Sweetness index | 0.000             | 0.000             |
| Astringency index | 0.000           | 0.389             |
| Total solids    | 0.002             | 0.219             |
| Solids non fat  | 0.000             | 0.014             |

If p-value < 0.05 means the factor has significant effect on parameter.
The pH values ranged from 3.85 to 4.37 in the yoghurt drinks samples. The statistical analysis also revealed a significant difference (p<0.05) among the yoghurt drinks samples in pH values observed (Table 3). The pH values decrease in increasing starter concentration and fermentation time. Sample A had a higher pH value (4.37) while sample F had a lower pH value (3.85). The pH values of yoghurt from buffalo milk in research fulfilled maximum standard pH of yoghurt based Food Standard Code, i.e., pH 4.5 [17].

The soluble solids values ranged from 7.25 to 9.90 in the yoghurt drinks samples. The statistical analysis also revealed a significant difference (p<0.05) among the yoghurt drinks samples in the soluble solids values observed (Table 3). The soluble solids values decrease in increasing starter concentration and fermentation time. Sample A had a higher pH value (9.90%) while sample F had a lower pH value (7.25). In the research, soluble solid is related to the acidity of yoghurt from buffalo milk that the more acidity yoghurt decrease soluble solid of yoghurt.

The sweetness and astringency indexes in the yoghurt drink samples were of the ranges from 7.86 to 15.94 and 0.06 to 0.13 respectively. The statistical analysis also revealed a significant difference (p<0.05) among the yoghurt drinks samples in sweetness and astringency indexes observed (Table 3). The astringency indexes indicated the level of lactic acid as a result of fermentation of lactic acid bacteria in yoghurt (Sanful 2009), so increasing acidity as well as increasing astringency indexes. Sample A had a higher sweetness index (15.94) while sample F had a lower sweetness index (7.86). A reverse result of astringency that sample A had a lower astringency index (0.06) while sample F had a higher astringency index (0.13). This showed that increased starter concentration and fermentation time resulted in increased astringency indexes.

The total solids values of yoghurt drink samples ranged from 13.20 to 15.40%. Total solid of yoghurt is yoghurt without water content or dry content of yoghurt. The total solid of sample A was highest (15.40%) in 3% starter concentration and 8 hours fermentation and the lowest was sample E (13.20%) in 5% starter concentration and 12 hours fermentation. It shows 5% starter concentration and 12 hours fermentation decrease in total solids. The statistical analysis revealed that starter concentration had a significant effect (p<0.05) on total solids observed, but fermentation time had no a significant effect (p>0.05) on total solids observed (Table 3). Solid non-fat is total solid without fat. The soluble solid values of yoghurt drink samples ranged from 5.45 to 11.49%. Sample A had a higher solids non-fat (11.49) while sample B had a lower solids non-fat (5.45%). It shows 5% starter concentration and 8 hours fermentation decrease in solids non-fat. The statistical analysis also revealed a significant difference (p<0.05) among the yoghurt drinks samples in solids non-fat observed.

3.2. Microbial content

The microbial contents of the yoghurt drink samples from buffalo milk observed are shown in Table 4.

| Yoghurt drinks of buffalo milk sample | A      | B      | C      | D      | E      | F      |
|--------------------------------------|--------|--------|--------|--------|--------|--------|
| LAB                                 | 7.78±0.42 | 7.88±0.51 | 8.11±0.24 | 8.10±0.12 | 8.18±0.12 | 8.22±0.17 |
| TPC                                 | 8.54±0.10 | 8.72±0.31 | 8.88±0.15 | 8.83±0.45 | 8.88±0.15 | 9.16±0.23 |

Table 5. The result of analysis statistical of yoghurt drink from buffalo milk’s microbial contents

| Parameters | p-value of factor |
|------------|-------------------|
| LAB        | 0.605             |
| TPC        | 0.252             |

If p-value < 0.05 means the factor has significant effect on parameter.
The results of lactic acid bacteria (LAB) of yoghurt drink from buffalo milk samples increased in increasing starter concentration but decreased in increasing fermentation time (Figure 1). The statistical analysis revealed no significant difference (p>0.05) among the yoghurt drinks samples on the lactic acid bacteria observed (Table 5). The total lactic acid bacteria ranged from 7.78 to 8.22 log cfu/ml. The highest LAB of the samples was in sample F (8.22 log cfu/ml) and the lowest LAB of the samples was sample A (7.78 log cfu/ml). Streptococcus thermophillus and Lactobacillus bulgaricus are lactic acid bacteria in yoghurt. The daily dose of Streptococcus thermophillus and Lactobacillus delbrueckii subsp bulgaricus in yoghurt were 8x1010 CFU/g, and the survival of Streptococcus thermophillus and Lactobacillus delbrueckii subsp bulgaricus were 6,3 x 10^4 and 7,2 x 10^4 CFU/g feces respectively after consuming yoghurt [6]. Lactic acid bacteria in yoghurt give beneficial effect for gut health because they can prevent tumorization caused by chemically carcinogens and enteric infection in the gastrointestinal tract [9].

![Figure 1](image1.png)

**Figure 1.** The lactic acid bacteria (LAB) of yoghurt drink from buffalo milk samples with starter concentration and fermentation time factors

![Figure 2](image2.png)

**Figure 2.** The total plate count (TPC) of yoghurt drink from buffalo milk samples with starter concentration and fermentation time factors

The results of total plate counts (TPC) of yoghurt drink from buffalo milk samples also increased in increasing starter concentration and fermentation time (Figure 2). The statistical analysis revealed no significant difference (p>0.05) among the yoghurt drinks samples on the total plate counts observed
The total plate counts ranged from 8.54 to 9.16 log cfu/ml. The highest LAB of the samples was in sample F (9.16 log cfu/ml) and the lowest LAB of the samples was sample A (8.54 log cfu/ml).

4. Conclusion
This results showed that starter concentration and fermentation time had a significant effect on acidity, pH, soluble solid, sweetness index, astringency index, and solid non-fat, but total solid had only a significant effect by starter concentration. Starter concentration and fermentation time had no significant effect on lactic acid bacteria and total plate count, so it means that 3% starter concentration starter and 8 hours of fermentation time had given enough good growth of lactic acid bacteria on yoghurt drink.

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