Effect of concentration starter and fermentation time on the quality of yoghurt drink from buffalo milk

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Abstract. Generally yogurt drinks are made from cow’s milk, whereas buffalo milk has a higher nutrition value than cow’s milk. The objective of this study was to produce yoghurt drinks from buffalo milk and determine proximate composition and sensory quality of the product based on starter concentration and fermentation time. The experiment was carried out using a completely randomized design of factorial patterns consisting two factors, i.e. starter concentration (3,5 and 10%) and fermentation time (8 and 12 hours). The results of proximate analysis obtained showed starter concentration had significant effect on moisture, fat, protein and carbohydrate contents. While fermentation time had significant effect on fat and protein contents. The sensory evaluation result showed significant differences among the yoghurt drink samples. Sample F (10% starter concentration and 12 hours fermentation time) had the highest score in sensory attributes.

1. Introduction
Buffalo milk has a higher nutritional content than cow’s milk. Protein and fat content in buffalo milk is quite high. Protein and fat content of cow’s milk are 3.4% and 4.0% respectively [1], compared to protein content and fat of buffalo river milk are 4.68% and 4.13% respectively. Buffalo milk contains essential amino acid and non-essential amino acids. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine that obtained from feed consumed by buffalo. The non-essential amino acids are aspartic acid, glutamic acid, serine, glycine, arginine, alanine and tyrosine. Furthermore, buffalo milk contains saturated and unsaturated fatty acids. Palmitic acid of 24.6%, stearic acid of 10.16%, myristic acid of 6.06% and lauric acid of 1.66% are saturated fatty acid in buffalo milk. Buffalo milk contains oleic acid of 16.3% and palmitoleic acid of 1.63% which are monosaturated fatty acids, while the levels of polyunsaturated fatty acids in buffalo milk are very little under 1% of total milk fat, namely omega 3 and omega 6 [2]. The amount and composition of fatty acids from milk fat can be influenced by several factors such as buffalo type, genetic, lactation period, mastitis and feed nutrition [3].

Buffalo milk is rich in minerals and vitamins that are important for human body. The minerals contained in buffalo milk are calcium (Ca) of 0.97-1.21 g/kg, phosphorus (P) of 0.59-0.76 g/kg, magnesium (Mg) of 0.06-0.08 g/kg, potassium (K) of 0.91-1.11 g/kg, sodium (Na) of 0.31-0.37 g/kg, copper (Cu) of 0.26-0.35 mg/kg, manganese (Mn) at 0.26-0.27 mg/kg, zinc (Zn) at 3.71-4.04 mg / kg and iron (Fe) at 1.37-1.58 mg / g [4]. Buffalo milk contains vitamin A at 340 IU / m, vitamin B1
Buffalo milk is sold in fresh and people of North Sumatera usually only process buffalo milk into a traditional food called dali or bagot ni horbo for consumption or resale. Although nutritional content of buffalo milk is quite high, community less interest to consume, so it is important to diversify buffalo milk to probiotic based food product like yoghurt drink from buffalo milk. Low-fat buffalo milk yoghurt contains a similar amount of protein, fat, carbohydrates, and calcium to cow’s milk yoghurt. Probiotic \textit{Lactobacillus acidophilus} on live buffalo milk yoghurt with an amount greater than $1 \times 10^6$ CFU/g for the first two weeks, whereas \textit{Bifidobacterium} spp. and \textit{Lactobacillus casei} persisted for ten weeks [11].

Making yoghurt drink is one of food diversification that aims to improve functional quality of buffalo milk, so that it is expected to increase public interest in consuming it. Some previous studies had shown the benefits of probiotic drinks. Some probiotic-based drinks are yoghurt that has antibacterial activity against \textit{Escherichia coli} and \textit{Bacillus cereus} [6], yoghurt drink with beet extract [7], fermented goat milk [8], pineapple juice lactate fermented drinks that have antagonistic activity against pathogenic bacteria \textit{Bacillus cereus} [9], yakult fermented milk that has the effect of improving the quality of bowel movements, \textit{Bifidobactera} in the intestine and intestinal health [10].

Therefore, this research aimed to make yoghurt drink from buffalo milk and discover the effect of starter concentration and fermentation time on proximate parameters (moisture, ash, protein, fat and carbohydrate contents) and sensory parameters (colour, aroma, taste and mouth feel).

2. Materials and methods

This research used fresh buffalo milk from Asam Kumbang in Medan and starter yoghurt in freeze-dried from North Jakarta. This research was conducted at Microbiology Laboratorium, Faculty of Agriculture, Universitas Sumatera Utara, Medan. The bacterias in starter yoghurt were \textit{Streptococcus thermophilus}, \textit{Lactobacillus bulgaricus} and \textit{Lactobacillus acidophilus}. The starter was activated by inoculated it to pasteurized milk and incubated in $37^\circ$C for 8 hours. It called work culture. The number of lactic acid bacteria in work culture was counted by MRSA media, that is $8.35 \pm 0.15 \text{ log cfu/ml}$. This research used completely randomized design of factorial patterns consisting two factors, i.e. starter concentration and fermentation time. The work cultures were inoculated to pasteurized buffalo milk with three different starter concentration, i.e. 3%, 5% and 10% as factor in the research. After that, they were incubated at $37^\circ$C with different fermentation time, i.e. 8 hours and 12 hours. After fermentation, the buffalo milk yoghurt obtained. Boiled water (1:1) and sugar 5% were added to buffalo milk yoghurts to result yoghurt drinks. The yoghurt drinks sample were labeled as samples A, B, C, D, E and F (Table 1).

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

The samples were cooled quickly to 10$^\circ$C in refrigerator for following analysis. The analysis used in research were proximate and sensory analysis. Proximate composition of yoghurt drinks samples were determined by SNI 01-2891-1992 method [12]. Proximate composition consists of moisture, ash, protein, fat and carbohydrate parameters. The moisture contents were determined by oven method. The ash contents were determined by the muffle furnace ignition method. The protein contents were determined by the formal titration method. The fat contents were determined by solvent extraction method. The carbohydrate contents were determined by iodometric titration. Sensory evaluation of the yoghurt drinks samples were performed by 20 panelist on 7 point hedonic scale for different parameters.
such as colour, aroma, taste and mouth-feel. Data obtained from proximate and sensory analysis of the samples were analysis statistically using a variance analysis (ANOVA).

3. Result and discussion

3.1. Proximate analysis of yoghurt drinks from buffalo milk
The result of proximate analysis from buffalo milk in this research were shown in Table 2. The moisture content of yoghurt drinks from buffalo milk ranged between 84.6 to 86.8% in all yoghurt drink samples. The starter concentration had significant effect (p<0.05) on moisture contents observed (Table 3). Figure 1 shows the highest moisture content was in sample E of 86.8%, i.e. yoghurt drink had 5% starter concentration and 12 hours fermentation time. While, the lowest moisture content was in sample A of 84.6%, i.e. yoghurt drink had 3% starter concentration and 8 hours fermentation time.

Table 2. Proximate analysis of yoghurt drink from buffalo milk

| Yoghurt drinks of buffalo milk samples | Parameters (%) | A     | B     | C     | D     | E     | F     |
|---------------------------------------|----------------|-------|-------|-------|-------|-------|-------|
| Moisture                              |                | 84.6±0.14 | 86.5±0.42 | 86.6±0.28 | 86.1±0.28 | 86.8±0.28 | 85.5±0.28 |
| Ash                                   |                | 0.45±0.07  | 0.48±0.04  | 0.44±0.03  | 0.49±0.08  | 0.46±0.04  | 0.43±0.01  |
| Protein                               |                | 2.64±0.13  | 3.06±0.14  | 2.89±0.06  | 3.15±0.14  | 3.47±0.17  | 3.32±0.10  |
| Fat                                   |                | 3.91±0.08  | 8.05±0.14  | 3.83±0.07  | 7.23±0.38  | 2.74±0.16  | 3.21±0.21  |
| Carbohydrate                          |                | 8.40±0.28  | 1.91±0.47  | 6.24±0.13  | 3.03±0.04  | 6.53±0.23  | 7.54±0.58  |

Data is the means of two replicates ± standard deviation

Table 3. The result of analysis statistical of yoghurt drink from buffalo milk

| Parameters     | p-value of factor |
|----------------|-------------------|
|                | Starter concentration | Fermentation time |
| Moisture       | 0.002              | 0.219             |
| Ash            | 0.587              | 0.917             |
| Protein        | 0.018              | 0.001             |
| Fat            | 0.000              | 0.000             |
| Carbohydrate   | 0.000              | 0.389             |

If p-value < 0.05 means the factor has significant effect on parameter

The ash content was between 0.43 to 0.49% in all yoghurt drink samples. The statistical analysis revealed that there were not significant differences in the ash contents observed (Table 3). But Figure 2 shows that in 12 hours fermentation the ash contents of yoghurt drinks decreased as the proportion of the starter concentration increased, with the highest ash content was in sample D (5% starter concentration) of 0.495 and the lowest ash content was in sample F (10% starter concentration).

The starter concentration and fermentation time factors gave significant effect (p<0.05) on protein contents (Table 3). The protein content was between 2.64 to 3.47% in all yoghurt drink samples. Figure 3 shows the protein content increased as the fermentation time increased. The highest protein content in sample E of 3.47%, that is yoghurt drink had 5% starter concentration and 12 hours fermentation time. While, the lowest protein content in sample A of 2.64%, that is yoghurt drink had 3% starter concentration and 8 hours fermentation time.
Figure 1. The moisture contents of yoghurt drink from buffalo milk samples

Figure 2. The ash contents of yoghurt drink from buffalo milk samples

The statistical analysis revealed that there were significant differences \((p<0.05)\) among the yoghurt drinks samples in the fat contents observed (Table 3). Figure 4 shows sample B (5% starter concentration and 8 hours fermentation time) had the highest fat content (8.05%), while sample E (5% starter concentration and 12 hours fermentation time) had the lowest fat content (2.74%). The fat content decreased as the fermentation time increased at 5% starter concentration.

Figure 3. The protein contents of yoghurt drink from buffalo milk samples

Figure 4. The fat contents of yoghurt drink from buffalo milk samples

The carbohydrate content was between the range 1.91 to 8.40%. The starter concentration had significant effect \((p<0.05)\) on carbohydrate contents observed (Table 3). Sample A (3% starter concentration and 8 hours fermentation time) had the highest carbohydrate content (8.40%), while sample B (5% starter concentration and 8 hours fermentation time) had the lowest fat content (1.91%). Figure 5 shows that the carbohydrate contents increased as the starter concentration increased at 12 hours fermentation.
3.2. Sensory analysis of yoghurt drinks from buffalo milk

The mean sensory scores of the organoleptic evaluation for the different yoghurt drinks samples are shown in Table 3. Sensory scores of the yoghurt drinks samples consisted of 7 point hedonic scales for different parameters such as colour, aroma, taste and mouth-feel, i.e. score 1 is very dislike, score 2 is dislike, score 3 is rather dislike, score 4 is neutral, score 5 is rather like, score 6 is like and score 7 is very like. The statistical analysis revealed that there were significant differences (p<0.05) among the yoghurt drinks samples in the sensory attributes observed (Table 5).

Yoghurt drinks with 10% starter concentration and 12 hours fermentation (sample F) resulted in better colour, aroma, taste and mouth-feel. Sample F had the highest scores of colour, aroma, taste and mouth-feel, i.e. 6.67, 6.23, 6.63 and 6.67 respectively, while sample A (yoghurt drinks with 3% starter concentration and 8 hours fermentation) had the lowest scores of colour, aroma, taste and mouth-feel, i.e. 5.10, 4.90, 5.17 and 5.23 respectively. The sensory scores in sample F related to acidity that gave better aroma and taste in yoghurt drink samples.

Table 4. Sensory analysis of yoghurt drink from buffalo milk

| Parameters of buffalo milk samples | A (%) | B (%) | C (%) | D (%) | E (%) | F (%) |
|-----------------------------------|-------|-------|-------|-------|-------|-------|
| Colour                            | 5.10±0.05 | 6.13±0.09 | 6.30±0.05 | 5.40±0.09 | 6.40±0.19 | 6.67±0.09 |
| Aroma                             | 4.90±0.14 | 5.47±0.09 | 5.80±0.19 | 5.23±0.14 | 6.10±0.05 | 6.23±0.14 |
| Taste                             | 5.17±0.24 | 6.23±0.05 | 6.43±0.14 | 5.90±0.14 | 6.50±0.05 | 6.63±0.05 |
| Mouth-feel                        | 5.23±0.05 | 6.17±0.05 | 6.37±0.05 | 5.63±0.33 | 6.47±0.19 | 6.67±0.19 |

Table 5. The result of analysis statistical of yoghurt drink from buffalo milk

| Parameters          | p-value of factor | p-value of factor |
|---------------------|-------------------|-------------------|
|                     | Starter concentration | Fermentation time |
| Colour              | 0.000              | 0.001             |
| Aroma               | 0.000              | 0.001             |
| Taste               | 0.000              | 0.002             |
| Mouth-feel          | 0.000              | 0.018             |

If p-value < 0.05 means the factor has significant effect on parameter
4. Conclusion
Proximate analysis of yoghurt drinks from buffalo milk obtained showed starter concentration had significant effect on moisture, fat, protein and carbohydrate contents. While fermentation time had significant effect on fat and protein contents. Sample E (5% starter concentration and 12 hours fermentation time) had the highest moisture and protein contents, i.e. 86.8±0.28% and 3.47±0.17% respectively. Sample D (3% starter concentration and 12 hours fermentation time) had the highest ash content, i.e. 0.49±0.08%. Sample B (5% starter concentration and 8 hours fermentation time) had the highest fat content, i.e. 8.05±0.14%. Sample A (3% starter concentration and 8 hours fermentation time) had the highest carbohydrate content, i.e. 8.40±0.28%. The sensory evaluation result showed significant differences among the yoghurt drink samples. Sample F (10% starter concentration and 12 hours) had the highest score in sensory attributes of colour, aroma, taste and mouth-feel, i.e. 6.67, 6.23, 6.63 and 6.67 respectively.

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