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The Effect of Fermentation Time on the Physicochemical and Microbiological Qualities of Buffalo Colostrum Kefir

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Abstract

This research aims to determine the best fermentation time to produce optimal buffalo colostrum kefir. Different fermentation times at 12, 24, 36, and 48 hours were used to measure physicochemical and microbiological characteristics of buffalo colostrum kefir. To produce buffalo colostrum kefir, fermentation process at room temperature (27±1 °C) was used. Viscosity was measured using an Ostwald viscometer and Total Dissolved Solid (TDS) was measured by digital refractometer. Titratable acidity was analyzed using the titration method and the pH value was measured using a pH meter. Protein content was analyzed using the Kjeldahl method. The results showed no significant differences (P>0.05) on viscosity which resulting in value of 0.80 to 0.92 cP. Meanwhile, significant differences (P<0.05) on TDS (13.30 to 24.02 °Brix), titrable acid (1.96 to 2.53%), pH value (3.37 to 3.95), protein content (10.57 to 14.41 %), total LAB ($2.85x10^6$ to $9.57x10^6$ CFU/ml), total yeast ($2.16x10^5$ to $7.76x10^5$ CFU/ml), and total microbes ($5.15x10^6$ to $5.21x10^6$ CFU/ml). The best treatment was 36 hours fermentation with 20% grain concentration (w/v) because it produced the highest total Lactic Acid Bacteria (LAB), total yeast, and total microbes, so it has the potential to be a probiotic drink.

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Introduction

Indonesian livestock as an agricultural sub-sector plays an important role in supplying food such as meat, eggs, and milk. One type of livestock commodity that is widely cultivated in Indonesia is buffalo. Buffalo milk has better quality than cow milk. Buffalo milk contains better macronutrients than cow's milk. Buffalo milk contains important minerals such as 0.152 mg/100 ml iron, 31.4 mg/100 ml sulphur, 184 mg/100 ml of total minerals, low fat (8%), and 340 IU/ml vitamin A (Garau *et al*., 2021). One of the important components contained in milk is colostrum.

Colostrum is a yellow fluid released by the mother of dairy cattle during lactation after giving birth for approximately 24 to 168 hours. The use of buffalo colostrum is currently still very limited and is only used for calves, even though colostrum contains various bioactive components such as immunoglobulins, antimicrobial substances, lysozyme, lactoferrin, and lactoperoxidase (Salehet *et al*., 2020). Buffalo colostrum also contains total solids and contains macro and micronutrients such as fat, lactose, phosphorus, calcium, magnesium, and vitamin E, which are higher than those in cow colostrum (El-Fattah *et al*., 2012). Buffalo colostrum has a slightly different nutritional content from cow colostrum. Buffalo colostrum contains 26.7 total soluble solids, 13.5 total protein, 9.9 fat, 2.4 total lactose, and 1.3 % ash. Cow colostrum contains 24.2 total soluble solids, 13.4 total protein, 8.0 fat, 1.7 total lactose, and 1.0 % ash concentration (Abd El-Fattah et al., 2014). The nutritional content and antimicrobial substances in buffalo colostrum make buffalo colostrum as a functional food product that can have benefits for the health of the human body. However, buffalo colostrum is still rarely used for food products due to the lack of public knowledge regarding the use of colostrum, and the ingredients are quite difficult to obtain because it is only found in buffalo mothers who have just given birth.

Based on the conditions existing in the farm, the excess colostrum produced by the parent livestock is not used properly by the breeder. Colostrum is actually processed into a product, namely milk tofu. However, there are errors in processing when cooking milk tofu, which results in good nutritional content in the colostrum. After the colostrum is processed into milk tofu, the milk tofu is fried using oil at high temperatures, even though the bioactive components in the colostrum will be damaged if heated above 60℃. Bioactive components that are damaged during high-temperature heating include immune substances such as immunoglobulins (IgG and IgM), IGF-1, and lactoferrin (Abd El-Fattah *et al.,* 2014). This condition causes processed colostrum to lose its nutritional value and important compounds. Therefore, buffalo colostrum has the opportunity to be processed into other products as a form of food diversification without losing the functional value of colostrum, which is beneficial for health.

Colostrum kefir is an alternative product that can be processed from buffalo colostrum. The use of buffalo colostrum is expected to produce good quality kefir, but research related to the optimal fermentation time for making buffalo colostrum kefir has never been conducted. Previously, research on colostrum kefir was conducted by (Ayar *et al*., 2016) related to the use of bovine colostrum in making kefir, so no research has been conducted on the use of buffalo colostrum with optimal fermentation time to produce good quality kefir. Previous research on cow colostrum kefir conducted by Windayani *et al*. (2020) using a kefir grain concentration of 20% (w/v) showed that antimicrobial activity increased with increasing titratable acidity and decreasing pH values. This research only shows the antimicrobial activity of colostrum kefir along with the length of fermentation time. Therefore, it is necessary to further test the chemical, physical and, microbiological characteristics colostrum kefir. This research also shows that fermentation time influences microbial activity in colostrum kefir. Microbial activity plays a significant role in the quality and characteristics of colostrum kefir. Therefore, further research is required on the effect of fermentation time on the characteristics of colostrum kefir.

Materials and Methods

Materials

Buffalo colostrum was obtained from dairy buffalo farming, Pampangan, Palembang, South Sumatra, Indonesia, and *grain* kefir was obtained from *Omah Kefir* Ungaran, Central Java, Indonesia. The chemicals used in this research were analytical reagent grade.

Method

The study was conducted from October to November, 2022. Buffalo colostrum is frozen at -20°C, then thawed by soaking in warm water (40°C) (Morrill *et al*., 2015). The tools were sterilized with boiling water. Grain kefir was added as much as 20% (w/v) of the total colostrum and put in a container so that fermentation can proceed. The kefir grain used previously had been tested for total bacteria using the Total Plate Count (TPC) method with Plate Count Agar (PCA) medium to determine the number of microbes contained in the starter. A good kefir starter for probiotic drinks is at least 10^6 CFU/ml. The treatments applied were different fermentation times, namely 12, 24, 36, and 48 hours in sealed jars at room temperature (27±1°C). After fermentation, the kefir was then filtered so that the kefir grains and the kefir can be separated.

Ostwald viscometer was used to determine viscosity (AOAC, 1990), digital refractometer was used to analyze Total Dissolved Solid (TDS) (AOAC, 2005), and pH meter was used to determine pH value (Ruck, 1963). The titration method was used to analyze titratable acidity (Ruck, 1963), the Kjeldahl method to analyze protein content (AOAC, 2005). Total Lactic Acid Bacteria (LAB) was identified using de Man Rogosa Sharpe Agar (MRSA) medium (Khedid *et al*., 2009), total yeast was identified using Sabaroud Dextrose Agar (SDA) medium and calculated using the Total Plate Count (TPC) method (Subramanya *et al*., 2017), and total microbes were identified using Plate Count Agar (PCA) medium and analyzed using the Standard Plate Count (SPC) method (Adams and Moss, 2008).

The obtained data were statistically analyzed using one-way ANOVA and Duncan's Multiple Range Test using SPSS 26.0. The level of significance was set at α = 0.05.

Results and Discussion

The viscosity of buffalo colostrum kefir was 0.80 to 0.92 cP (Table 1), resulting in no significantly differences on both values. The viscosity of buffalo colostrum kefir decreased slightly with the duration of fermentation. The viscosity of buffalo colostrum kefir products is related to the protein content in it because protein can bind to water molecules, thereby increasing the viscosity of the product (Putri *et al*., 2020). Buffalo colostrum kefir has a higher viscosity than its raw material, buffalo colostrum. This is because during the fermentation process there is a process of coagulation of proteins in colostrum due to changes in the atmosphere to become acidic due to the activity of lactic acid bacteria. The texture of kefir becomes thick because the protein in milk reaches the isoelectric point due to the acidic environment during the fermentation process so that the protein can coagulate (Erzhad *et al*., 2022).

Total Dissolved Solid (TDS) of buffalo colostrum kefir showed significant differences among fermentation times (Table 1). The highest TDS result was in 12 hours fermentation; i.e., 24.02 °Brix and the lowest was in 48 hours fermentation; i.e., 13.30 °Brix. The most dominant component in the total dissolved solids of buffalo colostrum kefir is lactose. According to Coroian *et al*. (2013), buffalo colostrum contains an average of 4.52% lactose. The total dissolved solids can be used to interpret the amount of sugar contained in the ingredients. Lactose is the most dominant sugar found in milk (Yap *et al*., 2017). The total dissolved solids of buffalo colostrum kefir decreased with the fermentation time. The decrease in total dissolved solids was caused by the activity of lactic acid bacteria, which break down lactose in buffalo colostrum into lactic acid. According to Chen *et al*. (2017), lactic acid bacteria use lactose to obtain energy and carbon. Lactose is broken down into simple sugars, namely glucose and galactose, using the help of the enzyme galactosidase which is then converted into pyruvic acid and lactic acid as the final product.

Table 1. Physicochemical Test Results for Buffalo Colostrum Kefir with Different Fermentation Times

Parameters	Fermentation Times (hours)			
	12	24	36	48
Viscosity (cP)	0.92±0.09	0.96±0.12	0.82±0.09	0.80±0.04
Total Dissolved Acid (°Brix)	24.02±0.80[d]	18.44±0.70[c]	16.63±0.96[b]	13.30±0.76[a]
Titratable Acid (%)	1.96±0.10[a]	2.25±0.05[b]	2.42±0.04[c]	2.53±0.04[d]
pH	3.95±0.03[a]	3.83±0.04[b]	3.68±0.04[c]	3.37±0.04[d]
Protein (%)	14.41±0.94[a]	13.17±0.84[b]	10.88±0.61[c]	10.57±0.68[c]

Results are mean±standard deviation (n = 20); Different superscript letters in the same row indicates the significant differences ($p < 0.05$).

The titratable acidity total of buffalo colostrum kefir showed significant differences among fermentation times (Table 1). The highest titratable acidity was in the kefir with 48 hours fermentation i.e., 2.53%. Total acid increases with the duration of fermentation. According to Sulmiyati *et al*. (2019), during the fermentation process, lactic acid bacteria present in kefir grains decompose lactose through the process of glycolysis, namely lactose is converted into pyruvic acid, which is then broken down into lactic acid. Kefir starter produces lactic acid, other organic acids, acetic acid, propionic acid, and butyric acid as a result of fermentation metabolism. According to Leite *et al*. (2013), acetic acid in kefir is a product of citrate metabolism. Acetic acid is produced through the oxidation of ethanol and is the end product of lactose metabolism by lactic acid bacteria and acetic acid bacteria. However, butyric acid and propionic acid are produced by the metabolic activity of *Lactococcus sp.* and *Leuconostoc sp.* (Bengoa *et al*., 2019).

The pH value of buffalo colostrum kefir showed significant differences among fermentation times (Table 1), with the highest value; i.e., 3.95 at 12 hours fermentation and lowest value; i.e., 3.37 at 48 hours fermentation. The pH value decreased with the duration of fermentation because of microbial activity, especially lactic acid bacteria in kefir. According to Cotârleț *et al*. (2019), during the fermentation process, lactic acid bacteria present in kefir grains convert lactose from colostrum as energy in the growth process, then produce metabolites, especially lactic acid, which lowers the pH of kefir. The low pH of kefir is related to the total acid produced. The pH of kefir is a representation of the accumulation of organic acids; that is, if the total acid content is higher, the resulting pH will be lower. Low pH is caused by the accumulation of organic acids, alcohol, and other volatile compounds produced by microbes in kefir (Setyawardani and Sumarmono, 2015).

Protein content of buffalo colostrum kefir showed significant differences among different fermentation times (Table 1). The highest protein content was in 12 hours fermentation i.e., 14.41. Protein levels in buffalo colostrum kefir decreased with the duration of fermentation. This can be caused by the metabolic activity of lactic acid bacteria. Lactic acid bacteria are categorized as proteolytic bacteria that have a complex proteolytic system, both for the growth of lactic acid bacteria themselves and for making a real contribution to the formation of flavors in fermented products (Brown *et al*., 2017). According to Coroian *et al*. (2013), buffalo colostrum contains about 5-8% protein. Specifically, the proteins contained in buffalo colostrum are casein and whey protein. The protein content of buffalo colostrum kefir is higher than the buffalo colostrum itself. This is in accordance with research (Setyawardani *et al*., 2020) which shows that the fermentation process could increase the total protein of a product because some LAB in kefir grain produced an enzyme that increased the protein level. Protein is one of the components that make up the microbes in kefir grains. Kefir contains a complete protein that is partially digested and can facilitate digestion. Amino acids in milk change during the fermentation process, and kefir contains higher levels of threonine, serine, alanine, lysine, and ammonia than milk. Kefir also contains other amino acids, such as valine, isoleucine, methionine, lysine, phenylalanine, and tryptophan (Arslan, 2015).

Total Lactic Acid Bacteria (LAB) of buffalo colostrum kefir can be seen on Table 2. Different fermentation times showed the lowest LAB total; i.e., $2.85x10^6$ CFU/ml at 12 hours fermentation and the highest LAB total; i.e., $9.57x10^6$ CFU/ml. LAB experienced growth from 12 to 36 hours, and began to decrease to 48 hours. LAB grows by using lactose in colostrum. According to Norberto *et al*. (2018), LAB present in kefir grains converts lactose through a hydrolysis process with the enzyme lactase into glucose and galactose. Glucose and galactose, which are monosaccharides, go through the process of glycolysis into pyruvic acid which is further reduced by BAL to become lactic acid. The more nutrients available, the higher the LAB population. The LAB population decreased during 48 hours fermentation, presumably because the nutrients contained in kefir had run out and the pH value was too low. A pH that is too low causes lactic acid bacteria that cannot tolerate high acidity to become dormant and even die (Montanuci *et al*., 2012).

The total yeast of buffalo colostrum kefir can be seen on Table 2. The highest total yeast was shown at 36 hours fermentation; i.e., 1.05×10^6 CFU/ml. Yeast growth started from 12 to 36 hours fermentation, and decreased at 48 hours. Yeast in kefir use lactic acid produced by lactic acid bacteria. Yeast species that utilize non-lactose components, such as *S. cerevisiae,* use lactic acid as a carbon source for their growth (Nejati *et al*., 2020). Yeast also obtains nutrients from the breakdown of glucose. According to Yıldız-Akgül *et al*. (2018) yeast is classified as a heterofermentative microbe, namely a microbe that can convert a substrate into more than one compound. Yeast (*Candida kefir*) utilizes glucose (simple sugar) to be converted into alcohol/ethanol and CO2. Initially, glucose is broken down into pyruvic acid and undergoes further processes, namely decarboxylation to acetaldehyde. Acetaldehyde is then reduced to ethanol/alcohol. CO_2 gas which is too high as the duration of fermentation, causes the yeast to experience growth that is not optimal (Laureys *et al*., 2018).

Table 2. Microbiological Test Results for Buffalo Colostrum Kefir with Different Fermentation Times

Parameters	Fermentation Times (hours)			
	12	24	36	48
LAB (CFU/ml)	2.85×10^{6a}	2.33×10^{7b}	3.29×10^{7c}	9.57×10^{6d}
Yeast (CFU/ml)	2.16×10^{5a}	8.62×10^{5b}	1.05×10^{6b}	7.76×10^{5b}
Microbes Total (CFU/ml)	5.15×10^{6a}	1.54×10^{7b}	2.91×10^{7b}	5.21×10^{6a}

Results are mean (n = 20); Different superscript letters in the same row indicates the significant differences ($p < 0.05$).

The total microbes of buffalo colostrum kefir can be seen on Table 2. Different fermentation times showed that the lowest total microbe total is 5.15×10^6 CFU/ml at 12 hours fermentation and the highest is 2.91×10^7 CFU/ml. The limit for the total number of microbes in fermented products according to CODEX STAN 243-2003 concerning fermented milk is a minimum of 10^7 CFU/ml. This indicates that the total microbes in the buffalo colostrum kefir product gain the standards. The longer the storage of fermented milk, the greater the work of microbes, and at the optimum temperature the activity of microbial metabolism will increase so that microbial growth becomes faster and the microbial population increases (Casarotti *et al*., 2014). The microbes in buffalo colostrum kefir consist of various types of lactic acid bacteria and yeast. This is because the kefir grains or starter used in the manufacture of buffalo colostrum kefir contain lactic acid bacteria and yeast. Lactic acid bacteria convert most lactose into lactic acid, acetic acid, and other organic acids. The yeast found in kefir grains converts lactose in buffalo colostrum into alcohol and CO_2 gas (Mendes-Ferreira and Mendes-Faia, 2020).

Conclusion

The longer the kefir fermentation time, the higher the total titrated acid. Meanwhile, the values of pH, viscosity, total dissolved solids, and protein. The microbiological characteristics of kefir showed an increase LAB, yeast, and microbes up to 36 hours of fermentation. The best treatment for fermentation time for buffalo colostrum kefir products is 36 hours.

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