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To cite this article: H Rizqiati *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **803** 012045

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## Chemical and microbiological properties of buffalo milk kefir with different starter concentrations

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**Abstract.** The research aims to determine the effect of kefir grain starter concentration on alcohol content, total dissolved solids and total buffalo milk kefir microbes and to find out whether these parameters are in accordance with predetermined standards. The study used a Completely Randomized Design (CRD) with treatments given were (T1) kefir grain starter concentration of 2.5%, (T2) 5% kefir grain starter concentration, (T3) kefir grain starter concentration of 7.5%, and (T4) concentration 10% kefir grain starter. The parameters observed were alcohol content, total dissolved solids and total microbes. Data from the test results were analyzed using the Analysis of Variance (ANOVA) test with a significance level of 5% and if there is a difference then proceed with the Duncan test. Kefir grain starter concentration has a significant effect ( $p < 0,05$ ) on alcohol content, total dissolved solids and total microbes. The more concentrations of kefir grain, the alcohol content and total microbes increase. The more concentration of kefir grain, the more total dissolved solids decreases, with the optimal amount of kefir grain starter concentration is 7.5%.

### 1. Introduction

Milk is one of the most nutritious food products that is obtained from milking animals like cow, buffalo, horse, goat, and camels [1]. Buffalo milk has a better quality of protein, fat, and also mineral content compared to cow milk, where protein, fat and mineral content in buffalo milk is higher compared to cow milk. Buffalo milk has 7 - 8% of fat content and 4.2-4.6% of protein content [2]. The use of buffalo milk is intended to make use of the abundant source of the buffalo milk, with one of the ways to overcome the fishy odor of the milk is through fermentation so that the taste of the milk is acceptable organoleptically by the community. The use of buffalo milk can also be processed into functional food products with many health benefits, such as kefir. Processing buffalo milk into kefir can increase the preference of the milk itself due to the flavour and aroma produced by the yeast contained inside the product [3].

Biochemically, kefir has an alcohol content, fat content, total dissolved solid, and also total bacteria that consist of yeast and lactic acid bacteria. Kefir is one of the products that produced alcohol, according to the agreement of the Indonesian Ulama Council (MUI) food and drinks that contains alcohol must not exceed 1%, so food and drinks that contain alcohol more than 1% is considered haram for consumption. Kefir is a fermented dairy product as a result of the activity from lactic acid bacteria and yeast which is made by adding kefir grain directly to the milk of both cow, goat, or even buffalo which has health benefits[4]. Kefir microbial content is very important to know in order to assess whether kefir product is in accordance to the functional food standards. Lactic acid



will give kefir a sour taste. Kefir grain is a bacterial colonies that are symbiotic with the other elements forming a dense matrix that consist of lactic acid bacteria and yeast, where kefir grains contains *Saccharomyces kefir*, *Lactobacillus*, *Lactococcus sp.* and *Streptococcus lactis*.

Some of the health benefits of kefir like that has been researched such as controlling cholesterol metabolism, as a probiotic, antitumor (animal research), antibacterial and able to modulate the body immune system [5]. Buffalo milk kefir quality can be assessed from several parameters such as chemical properties like alcohol and fat content, physical properties like total dissolved solids, also microbiological properties such as total microbe of the product. The purpose of the research was to determine the effect of different kefir grain concentration on alcohol content, total dissolved solids, and total microbes of buffalo milk kefir and also to determine whether those parameters were in accordance with the predetermined standards. The benefits of the research were to increase the added value of buffalo milk, food diversification, and availability of probiotic kefir functional food, also by knowing the exact kefir grain concentration will produced a good quality of kefir product and did not exceed the predetermined standards.

## 2. Material and Methods

This research was conducted using the Completely Randomized Design with 4 treatment and 5 repetition. The Material used in this study were fresh buffalo milk obtained from the island of Sumatra, kefir grains obtained from Ungaran, distilled water, 0.85% physiological NaCl, and Plate Count Agar Merck (PCA) media. Tools that were used in this study are hand refractometer (Atago, Japan), pycnometer, analytical balance (Shimadzu, Japan), oven (Mettler, Germany), laminar flow (Airtech, Japan), and incubators (Mettler, Germany).

### 2.1. Methods

#### 2.1.1. The Making of Buffalo Milk Kefir

The making of buffalo milk kefir begins with the preparation of fresh buffalo milk by measuring the needs of buffalo milk for each experiment. Kefir grain then added to the buffalo milk according to the treatment namely 2.5%, 5%, 7.5%, and 10% (w/v) and stirred until evenly distributed. Milk is then fermented inside a plastic jar covered with plastic wrap and the store at room temperature and in a light tight place. Fermentation process was carried out for 24 hours and then the kefir grain were filtered after the fermentation was complete [6].

#### 2.1.2. Parameter Analysis Methods

The parameters that were tested includes alcohol content conducted with pycnometer by converting the density of the alcohol using the alcohol density table [7], total dissolved solid using hand refractometer [6] by measuring the Brix degree, and total microbe test conducted using plate count method [8]. Data that were obtained from alcohol content, total dissolved solid, and total microbe test were analyzed using Analysis of Variance test (ANOVA) with significance level 5% and followed by Duncan Multiple Range Test (DMRT) if there is a significant effect.

## 3. Result and Discussion

### 3.1. Alcohol content

Alcohol content test results of the buffalo milk kefir with differences in the concentration of starter grain kefir can be seen in Table 1.

**Table 1.** Test Result of Alcohol Content on Buffalo Milk Kefir

Treatment	Alcohol Content (%)
T1	0.132 ± 0.046 <sup>a</sup>
T2	0.466 ± 0.094 <sup>b</sup>
T3	0.814 ± 0.058 <sup>c</sup>
T4	1.162 ± 0.156 <sup>d</sup>

<sup>a-d</sup>Different superscript shows a significance difference at 0.05

Based on Table 1. showed an increase in alcohol content of the product. Alcohol content in kefir product varies between 0.5 - 1.0 %, where alcohol content of the product is influenced by yeast and heterofermentative bacteria that produces ethanol [9]. Addition of kefir grain into kefir product cause an increase in alcohol content of the product itself, where the increase in yeast activity in kefir product will also produce high concentration of alcohol inside the product as a result of metabolism in order to obtain energy. The production of alcohol inside kefir is caused by the growth of yeast forming alcohol during the making process of kefir, where yeast has the ability to transformed pyruvic acid into alcohol during the glycolysis process [7]. In anaerobic situation yeast reduces pyruvic acid into acetaldehyde and created ethanol [10].

### 3.2. Total Dissolved Solid

Total dissolved solid of the buffalo milk kefir with differences in the concentration of starter grain kefir can be seen in Table 2.

**Table 2.** Test Result of Total Dissolved Solid on Buffalo Milk Kefir

Treatment	Total Padatan Terlarut ( <sup>o</sup> Brix)
T1	4.94 ± 0.089 <sup>a</sup>
T2	4.82 ± 0.179 <sup>b</sup>
T3	4.58 ± 0.000 <sup>c</sup>
T4	4.00 ± 0.386 <sup>c</sup>

<sup>a-c</sup> Different superscript shows a significance difference at 0.05

Based on Table 2. Test result of total dissolved solid on kefir showed a decrease, where total dissolved solid is measuring the amount of materials that is dissolved inside the solution. Total dissolved solid can be used to interpret the amount of sugar that is contained inside the product especially lactose, because lactose is a the most dominant sugar found in milk where lactose undergoes breakdown during the kefir fermentation process. Lactose breakdown process into glucose and galactose is done using the help of enzyme that is produced by the kefir grain [6]. Degraded lactose into glucose and galactose which eventually will become lactic acid. Those changes caused by the activity of lactic acid bacteria. Microorganism activity is indicated by the decrease of sugar content, because microorganism growth can be optimal by requiring more sugar to be broken down so that there is less sugar left in the solution [11]. Kefir total dissolved solids standard is not yet been spesifically regulated by the Indonesia National Standard, thus in determining the total dissolved solid of kefir can use the standard for other fermented milk like yogurt, where Indonesia National Standard requires the minimum content of non-fat total dissolved solid is 8.2% [12].

### 3.3. Total Microbes

Total Microbe content of the buffalo milk kefir with differences in the concentration of starter grain kefir test result can be seen in Table 3.

**Table 3.** Test Result of Total Microbes on Buffalo Milk Kefir

Treatment	Total Microbes (CFU ml <sup>-1</sup> )
T1	5.93 x 10 <sup>6</sup>
T2	1.58 x 10 <sup>7</sup>
T3	6.62 x 10 <sup>7</sup>
T4	1.34 x 10 <sup>8</sup>

Based on Table 3. Test result of total microbes showed an increase, where the higher of the grain kefir concentration that is added to the product the higher the growth of the microbe. The result of

ANOVA data analysis shows a significant effect ( $p < 0.05$ ) between fermentation time and the total buffalo milk kefir microbes. The increase in total microbes is caused by the activity of yeast or microbe and minimum temperature that produces alcohol resulting in the decrease of kefir total acidity, where the increase of microbial activity and the increase of total microbe will cause more and more substrate being fermented [13]. The availability of raw material for making kefir is one of the factors that can influence the numbers of microbes, where if the availability of raw material is sufficient will cause microbial growth in kefir to increase. The standard amount of total microbe content in fermented milk is a minimum of  $10^7$  CFU/ml [14].

#### 4. Conclusion

Kefir grain concentration has an influence on alcohol content, total dissolved solid, and total microbe. The greater the kefir grain concentration, the higher the alcohol content and the higher the total microbe. The greater the kefir grain concentration, the lower the total dissolved solid content of the product. The use of the optimal starter concentration in the manufacture of buffalo milk kefir is a starter concentration of 7.5%.

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