

## Microbial Test: Combination of Kefir Milk, Star Fruit, and Red Ginger

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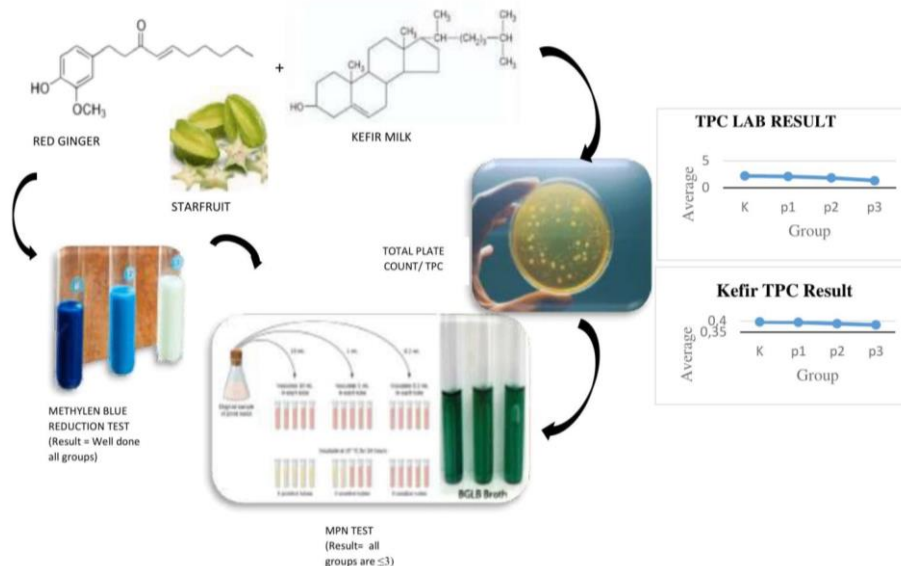
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### Abstract

One of drinks with high nutritional value and good for health is milk kefir. The advantage of milk kefir compared to yogurt (another fermented beverage) they are richer with probiotics. Goat's milk kefir contains more nutrients than cow's milk. To increase the production of sweet star fruit plants, the innovation of kefir milk drink combined with sweet star fruit juice and red ginger juice can be used as an alternative health drink that can increase body immunity. Each ingredient has the same benefits such as having antioxidant, antibacterial, anti-inflammatory, antiviral and immunomodulatory activities. In this study, researchers wanted to know the microbiological quality of the combination of milk kefir, sweet star fruit juice and red ginger. The study was conducted from February to May 2021. Microbiological tests carried out were Methylene blue test, MPN test and total plate count test. The research design used a completely randomized design which focused on one factor, namely the concentration of red ginger extract (control concentration 0%, 4.5%, 6%, 7.5%). This experiment was used with replicas 3 times. The results showed that kefir fermented goat milk with the addition of sweet star fruit juice and ginger juice was good for consumption, namely in treatment group 1 (containing 20% star fruit juice and 4.5 percent red ginger juice).

**Keywords:** kefir, star fruit, red ginger

### Graphical Abstract



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## Introduction

Kefir milk is a fermented milk product that utilizes the activity of Lactic Acid Bacteria (LAB), yeast, and acetic acid bacteria<sup>[1,2]</sup>. Kefir milk has been known in the Middle East for many years. This drink originated from the Caucasus mountains in the former Soviet Union, Central Asia, and has long been consumed by people at that time. The term "Kefir" is taken from the Turkish language, Kef (Keyif), which refers to goodness, fitness, and health<sup>[3,4]</sup>. The content of kefir milk compared to other fermented milk, such as yogurt, has been proven to have more benefits as well as nutrition and higher nutritional value. Kefir milk is thinner and yogurt is softer<sup>[5,6]</sup>. The milk ingredients that can be processed originate from goats and cows. Several studies have revealed that goat's milk is superior to cow's milk and has distinctive sensory characteristics, such as a strong goat aroma originating from short-chain fatty acids<sup>[7,8]</sup>. While the ingredient "Kefir" is a natural probiotic that contains vitamins, minerals and essential amino acids that help in healing and maintaining body functions. In addition, it also contributes to immunomodulatory, antibacterial, antitumor, healing of metabolic diseases, tuberculosis, and digestive tract diseases<sup>[9,10]</sup>. The weakness of goat kefir milk is due to its pungent aroma and less sweet taste, so many people are not interested in consuming it<sup>[11,12]</sup>.

Currently, in Indonesia, many researchers are developing herbal drinks and natural ingredients as processed food and health drink products as well as boosting immunity. These ingredients can originate from the fruits and spices<sup>[13,14]</sup>. However, the use of these natural materials can increase the number of commodities in an area. One of the areas in Indonesia that has the potential for this natural product is the Tulungagung-East Java area, especially in Moyoketen, which is known as a producer of sweet star fruit cultivation<sup>[15][16]</sup>. Sweet star fruit contains many nutrients and benefits, including antioxidant and antibacterial activity. The nutritional content of star fruit can be used as a prebiotic and nutritional supplement for the normal flora in the body. If star fruit is mixed with

kefir milk, it adds freshness, increases antioxidants, and increases the sale value<sup>[3,17]</sup>.

Some species are also being studied for their properties that can boost the body's immune system, one of which is red ginger. The content of red ginger is that there are high antioxidant compounds, anti-inflammatory, anticancer and also antibacterial. Recently, several studies have stated that adding spices such as ginger to milk can be used as a natural preservative for the structure of milk, can saving the quality of the milk. Therefore, researchers wanted to determine the quality of the combination of kefir milk compounds with starfruit juice and red ginger extract in vitro<sup>[18,19]</sup>.

Based on the background above, the researcher wanted to know the effect of the combination of kefir milk with starfruit juice and red ginger extract on health. The purpose of this study was to provide information on the bacteriological quality of the combination of kefir milk with belimbing fruit juice and red ginger juice.

## Experimental Section

### Materials

The materials used were goat milk from goat breeders in Pakel-Tulungagung Village, Belimbing fruit from Belimbing Tourism Moyoketen-Tulungagung, red ginger, starter, skimmed milk, distilled water, ethanol, NA media, SDA media, MRSA media, NaOH, HCl 0.9% NaCl KI, Amylum, KIO<sub>3</sub>, H<sub>2</sub>C<sub>2</sub>O<sub>4</sub>, methylene blue, and Gram paint.

### Instrumentation

The tools needed for this research were a petri dish, beaker glass, stir bar, aluminum foil, filter paper, jar, oven, incubator, spirit lamp, measuring flask, Erlenmeyer, balance, electric/gas stove, magnetic stirrer, pH, shaker rotator, centrifuge, and viscometer.

### Procedure

#### ***Milk reduction test with Methylene blue***

The reductase test was used to estimate the number of bacteria in milk using Methylene Blue (MB) substance. The function of MB is to give milk

a blue color because of the ability of bacteria to reduce milk. The faster the reduction time, the greater the number of bacteria in the milk (Kuswiyanto, 2016). In this study, 15 test tubes were used: positive control tube 1 (goat milk + 20% starfruit juice + 0% ginger without MB, tube 2 containing kefir milk + 20% starfruit juice + 0% ginger + MB, tube 3 containing kefir milk + 20% star fruit juice + 4.5% ginger + MB, tube 4 containing kefir milk + 20% starfruit juice + 6% ginger + MB, tube 5 containing kefir milk + 20% starfruit juice +7, 5% ginger + MB. Repeat 3 times. Color change was observed every 1–8 h.

**Most probability number (MPN) test**

MPN was assessed based on the estimated growth unit (GU) or colony-forming unit (CFU). There were three types of variances in the MPN test: Variety 5.1.1, Variety 5.5.5, and Variety 3.3.3. In the study using Variety 5.1.1, the test materials were processed. Several MPN tests include estimator, reinforcement, and complementary tests [20].

**Total plate count (TPC) test**

The Total Plate Count (TPC) test was used to count the number of microorganisms (bacteria/mold/yeast) in a 1 ml sample or 1 g sample. The principle of this examination was to directly inoculate the sample in ml/gram which was incubated for a certain and appropriate time; then, the number of colonies was counted visually [20].

**Results and Discussions**

**Milk reduction test with Methylene blue**

Based on the results of these observations, it is known that the quality of kefir milk is good in the absence of treatment, and there is treatment that shows the same results. In the first hour until the seventh hour it is clear that the clear blue and white color at the bottom of the tube is due uneven mixing of the samples. Then, only after the eighth hour did the blue color start to fade.

**Tabel 1.** Methylene blue reduction test results.

Sample	Initial state	Color Change (hours)								Milk Quality
		1	2	3	4	5	6	7	8	
control	Blue	Blue	Blue	Blue with a bit of white at the bottom of the tube	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Off blue with basic white	Well Done
45%	Blue	Blue	Blue	Blue with a bit of white at the bottom of the tube	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Off blue with basic white	Well Done
60%	Blue	Blue	Blue	Blue with a bit of white at the bottom	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Off blue with basic white	Well Done

				of the tube						
75%	Blue	Blue	Blue	Blue with a bit of white at the bottom of the tube	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Like the 3 <sup>rd</sup> hour	Off blue with basic white	Well Done

**Tabel 2.** Average MPN test results.

Group	MPN Value (cfu/ml)	Value Standard
K	0	≤3
P1	0	≤3
P2	0	≤3
P3	0	≤3

**Tabel 3.** Average BAL and kefir TPC values.

Group	R1	R2	R3	Average	STDEV
K	2.181	2.231	2.225	2.212	0.022
P1	2.103	2.1	2.103	2.102	0.001
P2	1.827	1.855	1.827	1.836	0.013
P3	1.329	1.344	1.329	1.334	0.007

In accordance with milk quality standards, the results of the above research included high-quality types of processed milk [10]. This is in line with the research of Lindawati (2015) and Pradana (2018) which states that the growth of pathogenic bacteria will be inhibited by LAB which is acidic so that the pathogenic bacteria unable to grow for a long time (I Putu Esa Pradana, Sri Sinto Dewi, 2014; S. A. Lindawati, N. L. P. Sriyani, M. Hartawan, 2015).

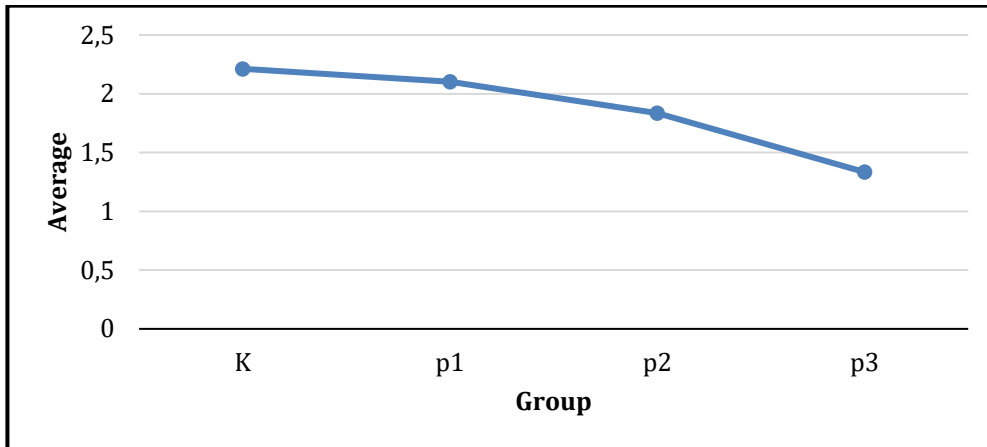
**Most probability number (MPN) test**

The following is Table 2. The average results of the Most Probability Number (MPN) Test. Based on Table 2 it is known that the MPN values in all groups are declared 0 and according to the MPN standard ≤3.

**Total plate count (TPC) test**

**BAL total plate count (TPC) test**

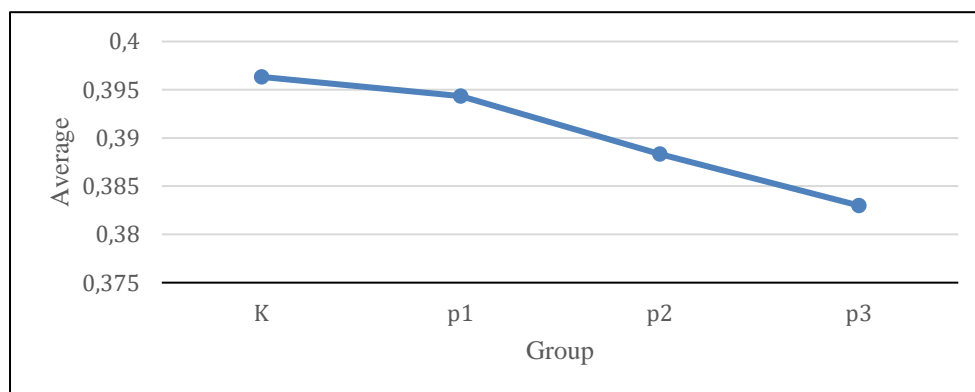
Based on Table 3, it is known that the lowest number of TPC BAL results was in treatment group 3 (P3), namely the kefir milk sample that received an additional 20% starfruit juice and 7.5 red ginger. The highest number was in the control group (K), where only kefir milk without the addition of star fruit juice or ginger juice was used. The normal distribution for each group was set at p>0.05. In this study, ginger extract did not significantly affect LAB activity, even though there was a decrease in the amount of LAB.



**Figure 1.** Graph of TPC BAL (Lactic Acid Bacteria) test results in CFU units.

**Tabel 4.** Average kefir TPC values.

Group	R1	R2	R3	Average	STDEV
K	0.394	0.42	0.375	0.396	0.018
p1	0.394	0.387	0.402	0.394	0.006
p2	0.398	0.388	0.379	0.388	0.007
p3	0.392	0.368	0.389	0.383	0.011



**Figure 2.** Graph of Kefir TPC test results.

**Kefir total plate count (TPC) test**

Based on Table 4. it is known that the lowest number of Kefir TPC results was in treatment group 3 (P3). namely the kefir milk sample that had received an additional 20% starfruit juice and

7.5 red ginger. And the highest number was in the control group (K) where it was only kefir milk without the addition of star fruit juice or ginger juice. The amount of kefir around 2.7%. The normal distribution for each group was set at  $p >$

0.05. In this study, we found that ginger extract did not significantly affect the activity of Kefir bacteria. even though there was a decrease in the amount of Kefir.

## Conclusions

The results of the quality test of fermented goat's milk with the addition of starfruit juice and ginger juice were obtained as follows: MPN with a value of 0/ml  $\leq$ 3, Methylene Blue Test with good results; and in TPC, there was a slight decrease in BAL and Kefir as the concentration of ginger increased. although not significantly. The amounts of LAB and Kefir in the treatment group were high in treatment group 1 (Ginger extract 4.5%). Therefore. It can be concluded that fermented goat milk with the addition of starfruit juice and ginger extract is good for consumption. particularly in treatment group 1 (containing 20% starfruit juice and 4.5% red ginger juice).

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