Production and characterization of synbiotic kefir cow milk with addition of *Dioscorea esculenta* puree

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Abstract. Kefir is an acidic, alcoholic, and carbonate-flavored milk product, which is widely consumed in the Caucasian region. Kefir is produced through natural fermentation of milk from cows, goats, or sheep with a combination of lactic organisms and yeast on kefir seeds or kefir grains. The type of bacteria and yeast used in the manufacture of kefir is *Lactobacillus* sp. and *Saccharomyces*. Kefir is a natural probiotic and is classified as a functional food. In addition, a well-known functional food is synbiotic, which is a combination of probiotics and prebiotics. One agricultural commodity that contains prebiotics is *Dioscorea esculenta*. In this research, synbiotic cow milk kefir was made by adding *Dioscorea esculenta* puree which aims to get a superior kefir product by adding *Dioscorea esculenta* puree to kefir, with the parameters of titrated acid total, pH value, viscosity, and growth of lactic acid bacteria and yeast. The results showed that the selected synbiotic kefir formulation was kefir with the addition of 8% *Dioscorea esculenta* puree with a fermentation time of 28 hours and had the following characteristics: titrated acid total 1.07%, pH value 3.87, viscosity 1231.37cp, lactic acid bacteria total 10.13 log CFU / ml and yeast total 9.08 log CFU / ml. The results of this analysis had fulfilled the CODEX standard No.243 of 2003.

Keywords: *Dioscorea esculenta*, kefir, probiotic, prebiotic, synbiotic

1. Introduction

Kefir is a fermented pasteurized milk product used as a starter in the form of a kefir seed (kefir grain / kefir granule). Kefir has a sour taste and is slightly aromatic with alcohol. Kefir is included in functional food because kefir contains bioactive components that are beneficial to human health. Kefir is a highly nutritious drink with relatively low milk sugar (lactose) content compared to whole milk [1]. Kefir is very useful for patients with lactose intolerant or not resistant to lactose, because the lactose has been digested into glucose and galactose by lactase enzymes from microbes in kefir seeds. Kefir has a higher probiotic content compared to yogurt and other fermented milk, and has many health benefits such as tuberculosis treatment, cancer, digestive disorders [2] [3] and as an anti-tumor [4].

Synbiotic drinks are products that contain both prebiotics and probiotics and have good effects on health. The availability of prebiotics in synbiotic drinks can help prevent colon cancer because it helps remove toxins produced by bacteria and prevents inflammation of the large intestine. The availability of prebiotics can be a source of carbon for probiotic bacteria so that it has better viability so that it...
functions better in the human body [5]. One agricultural commodity that contains prebiotics is *Dioscorea esculenta*. *Dioscorea esculenta* contain about 14% inulin [6][7][8]. Inulin is a carbohydrate that functions as an effective prebiotic, which is defined as a food component that cannot be digested by digestive enzymes so that it reaches the large intestine without undergoing structural changes and can selectively stimulate the growth and activity of beneficial bacteria in the digestive system [9]. Although kefir has a lot of benefits, but it is not as popular as other fermented milks such as yogurt. Kefir has a texture that separates solids and liquid like stale milk. Therefore, in this study a symbiotic cow's milk kefir was made by adding *Dioscorea esculenta* puree which aims to improve the quality of kefir which includes titrated acid total, pH value, viscosity, lactic acid bacteria total, and yeast total.

2. Experimental method

This research was conducted from February to August 2018 at the Surfactant and Bioenergy Research Center (SBRC) Laboratory LPPM IPB University, and the Agro-industrial Technology Department DIT Laboratory, FATETA IPB University. This research consists of 2 stages, namely the preparation of *Dioscorea esculenta* puree and cow milk kefir preparation with the addition of *Dioscorea esculenta* puree. *Dioscorea esculenta* used in this study comes from Kuningan, West Java Indonesia. *Dioscorea esculenta* puree is obtained through several stages of the process. First, fresh *Dioscorea esculenta* are sorted and washed, steamed for 45 minutes, peeled, then crushed using a blender. Preparation of synbiotic kefir is done by mixing fresh cow's milk and *Dioscorea esculenta* puree as much as 4%, 6%, and 8%. 1 liter of fresh cow's milk added by 40, 60, and 80 grams of *Dioscorea esculenta* puree, pasteurized at 90-95 °C for 5-10 minutes, then cooled to room temperature until it reaches 25 °C after which 5% kefir grain is added aseptically. Milk is incubated at room temperature (20-25 °C) for 20, 24 and 28 hours. Then it is proceeded with the analysis of titrated acid total, pH value, viscosity, lactic acid bacteria total and yeast total every 4 hours at 20, 24 and 28 hours. This study uses a Completely Randomized Design consisting of two factors, namely concentrations of *Dioscorea esculenta* puree (4%, 6%, and 8%) and fermentation time (20, 24, and 28 hours) with 3 repetitions. The data obtained are then analyzed with a completely randomized design of two factors (time and concentration) using SAS software version 9.4. Treatments that show significant differences in their effect on the observed response are further analyzed using the Tukey-Kramer test.

3. Result and discussion

3.1. Titrated acid total and pH value

In kefir fermentation, the lactose in milk is converted to lactic acid which makes kefir pH lower and acidic. Standard CODEX No.243 requires titrated acid total in kefir at least 0.6%.

![Figure 1. Graph of titrated acid total synbiotic kefir during fermentation time.](image1.png)

![Figure 2. Graph of pH value synbiotic kefir during fermentation time.](image2.png)

From the results of statistical analysis, the total value of titrated acid and kefir pH of synbiotic cow milk with the addition of *Dioscorea esculenta* puree with concentrations of 4%, 6%, and 8%, with a
fermentation time of 20 hours, 24 hours, and 28 hours, p-value for time and the concentration of Dioscorea esculenta puree obtained is less than 0.05. This means that the treatment influences the response tried at the 5% significance level. The interaction between time and concentration of Dioscorea esculenta puree also influences the 5% significance level. Then proceeds with the Tukey-Kramer test on the time and concentration of Dioscorea esculenta puree. The results of these treatments have different effects. The longer the fermentation time and the higher the concentration of Dioscorea esculenta puree added, the more it will increase the total value of titrated acid and decrease the pH value of synbiotic kefir. As time goes by fermentation, the number of starters or bacteria contained in kefir increases. With the increase in the number of starters, it increases the amount of lactic acid because microorganisms in the kefir starter increase the amount of lactic acid in kefir [10], which in turn also reduce the pH value. The total value of titrated acid in synbiotic cow milk kefir with the addition of tuber puree with concentrations of 4%, 6%, and 8%, with a fermentation time of 20 hours, 24 hours, and 28 hours has also fulfilled the CODEX standard No.243 of 2003 that the minimum titrated acid total contained in kefir is 0.6%.

3.2. Viscosity
During the kefir fermentation process, addition of Dioscorea esculenta puree to synbiotic kefir increases the thickness of the product. In addition, the presence of coagulation of milk protein caused by the production of lactic acid in kefir, also makes the synbiotic kefir texture produced becomes thicker.

From the results of statistical analysis, viscosity of synbiotic cow milk kefir with the addition of Dioscorea esculenta puree with concentrations of 4%, 6%, and 8%, with a fermentation time of 20 hours, 24 hours, and 28 hours, p-value for the time and concentration of Dioscorea esculenta puree obtained is less than 0.05. This means that the treatment influences the response tried at the 5% significance level. The interaction between time and concentration of Dioscorea esculenta puree also influences the 5% significance level. Then proceeds with the Tukey-Kramer test on the time and concentration of Dioscorea esculenta puree. The results of these treatments have different effects, with the treatment that most influences the response is the treatment with the highest average of 28 hours and 8% treatment. The longer the fermentation time and the higher the concentration of Dioscorea esculenta puree added, the more it increases the viscosity of synbiotic kefir. The longer the fermentation time, the more numerous the milk protein that clots due to the acidic conditions becomes, so kefir becomes thicker.

The addition of Dioscorea esculenta puree also affects the viscosity or thickness of the synbiotic kefir, the higher the concentration of the Dioscorea esculenta puree added, the further it increases the viscosity of the synbiotic kefir. This is because the Dioscorea esculenta contains water soluble polysaccharides. Water soluble polysaccharides are water soluble food fibers that are defined as
components in plants that are not degraded enzymatically into sub units that can be absorbed in the stomach and small intestine. Water soluble polysaccharides, commonly called hydrocolloid, is now widely used in the food industry, in order to achieve the desired quality, in terms of viscosity, stability, texture, and appearance [11]. The water soluble polysaccharides from the Dioscorea group contains the main glucomannan [12]. In addition, carbohydrates contained in Dioscorea esculenta are inulin and dietary fiber. Inulin contained in Dioscorea esculenta is 14.2% and dietary fiber is 2.6% [8]. The addition of Dioscorea esculenta puree to synbiotic kefir containing inulin and dietary fiber also adds to the thickness of the resulting kefir product.

3.3. Lactic acid bacteria total and yeast total
During the kefir fermentation process, lactic acid bacteria total and yeast total increases. This is due to the addition of substrate in the form of Dioscorea esculenta puree as a source of nutrition for bacteria and yeast in kefir.

![Figure 4. Graph of lactic acid bacteria total on synbiotic kefir during fermentation time](image)

![Figure 5. Graph of yeast total on synbiotic kefir during fermentation time](image)

From the results of statistical analysis of lactic acid bacteria total and yeast kefir total of synbiotic cow milk with the addition of Dioscorea esculenta puree with concentrations of 4%, 6%, and 8%, with a fermentation time of 20 hours, 24 hours, and 28 hours, p-value for the time and concentration of Dioscorea esculenta puree obtained is less than 0.05. This means that the treatment influences the response tried at the 5% significance level. The interaction between time and concentration of Dioscorea esculenta puree also influences the 5% significance level. Then proceeds with the Tukey-Kramer test on the time and concentration of Dioscorea esculenta puree. The results of these treatments have different effects, with the treatment that most influences the response is the treatment with the highest average of 28 hours and 8% treatment.

4. Conclusion
The addition of Dioscorea esculenta puree has an increased effect on the growth of lactic acid bacteria and yeast in the synbiotic kefir fermentation process. The results shows that the best synbiotic kefir formulation is kefir with the addition of 8% of Dioscorea esculenta puree with a fermentation time of 28 hours and has the following characteristics: titrated acid total 1.07%, pH value 3.87, viscosity 1231.37cp, lactic acid bacteria total 10.13 log CFU / ml and yeast total 9.08 log CFU / ml. The results of this analysis have met CODEX No.243 2003 standards.

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