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Impact of Golden Banana Puree (*Musa acuminata*) Enrichment on the pH, Lactic Acid Bacteria Count, and Viscosity of Kefir

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ABSTRACT. The addition of golden banana puree (*Musa acuminata*) as a prebiotic has been hypothesized to enhance the growth of lactic acid bacteria in kefir. This study aimed to determine the effects of adding golden banana puree on the pH value, total lactic acid bacteria (LAB) count, and viscosity of kefir. The experiment was conducted using a Completely Randomized Design (CRD) with four treatments and three replications. The treatments included the addition of 0% (P0), 6% (P1), 8% (P2), and 10% (P3) banana puree. The observed variables were pH value, total LAB, and viscosity. Data were analyzed using Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) when a significant treatment effect was detected. The results indicated that the addition of golden banana puree had a very significant effect ($P<0.01$) on the pH value, total LAB count, and viscosity of kefir. The study concluded that increasing the concentration of golden banana puree leads to a higher total LAB count and viscosity, while inversely lowering the pH value.

Keywords: golden banana puree, viscosity, kefir, pH value, total LAB

INTRODUCTION

In recent years, particularly following the Covid-19 pandemic, there has been an increased public awareness of health and nutrition. This has led to a growing demand for products that offer not only nutritional benefits but also positive health effects, commonly referred to as functional foods (Martharini and Indratiningsih, 2017). Kefir, a fermented drink with a distinctive sour and slightly alcoholic taste and a creamy consistency, is one such functional food (Yousefvand et al., 2022). Originating from the Caucasus and Tibet (Rosa *et al*., 2017), kefir is produced by fermenting milk (cow, goat, or sheep) with a mix of lactic acid bacteria (LAB), acetic acid bacteria, and yeast present in kefir grains (Ningsih *et al*., 2018). Kefir is known for its probiotic properties and is easily digestible (Łopusiewicz *et al*., 2022).

Kefir grains contain a complex community of microorganisms, including LAB such as Lactobacillus spp., Leuconostoc spp., Streptococcus spp., and Lactococcus spp., as well as yeasts like Saccharomyces spp., Kluyveromyces spp., Zygosaccharomyces spp., Torulopsis spp., and Candida spp., and possibly acetic acid bacteria (Acetobacter spp.) (Rohmah and Estiasih, 2018).

Kefir is classified as a functional food due to its bioactive components, which offer various health benefits. These include the presence of beneficial bacteria, vitamins, minerals, and essential amino acids that contribute to overall health and well-being (Kartika *et al*., 2020; Julianto *et al*., 2016). Research has shown that kefir can have positive effects on various health conditions, including colorectal cancer, cardiovascular disease, type 2 diabetes mellitus, obesity, and kidney disease (Yilmaz *et al*., 2022). Additional benefits include reducing inflammatory bowel disease severity (Yilmaz *et al*., 2019), exhibiting anticarcinogenic properties (Sharifi *et al*., 2017), improving serum lipid profiles (Fathi *et al*., 2017), and mitigating neurodegenerative diseases (Ton et al., 2020). Kefir is also used in cosmetic applications for skin lightening, anti-acne treatments, wound healing, and as an antioxidant (Dewi *et al*., 2018).

Despite its numerous benefits, kefir's consumption is often limited by its sour taste. To enhance its appeal, it is beneficial to explore the incorporation of other ingredients. Golden banana (Musa acuminata), which is rich in nutrients and acts as a prebiotic, could be an ideal candidate for this purpose. Golden banana, a monocot plant thriving in tropical regions such as Indonesia (Puguh *et al*., 2020), has high production yields, contributing significantly to the fruit market in Indonesia (Junior and Hariyono, 2020). Known for its round shape, bright yellow skin, and sweet flavor, golden banana offers several health benefits. According to the Indonesian Food Composition Table (TKPI) (2019), it contains essential nutrients including carbohydrates, fiber, vitamins, and minerals. It is particularly noted for its potential to reduce LDL cholesterol and increase HDL cholesterol, thereby improving cardiovascular

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health and reducing hypertension risk (Badriah *et al*., 2019). However, challenges related to its short shelf life and handling necessitate research into incorporating golden banana puree into kefir production.

This study aims to evaluate the effects of adding golden banana (Musa acuminata) puree on the pH value, total lactic acid bacteria (LAB) count, and viscosity of kefir.

MATERIALS AND METHODS

Material

The materials used in this study included pure milk sourced from dairy farms in Enrekang Regency, commercially obtained kefir grains, golden bananas, MRSA, distilled water, 70% alcohol, and other laboratory supplies such as tissue and label paper. The equipment consisted of a pan, stove, measuring cup, thermometer, digital scale, petri dish, containers, 200 and 400 mesh filters, pH meter, incubator, pycnometer, blender, and stirrer.

Method

The research employed an experimental approach using a Completely Randomized Design (CRD) with four treatments and three replications. The treatments were as follows: P0 (0% golden banana puree), P1 (6% golden banana puree), P2 (8% golden banana puree), and P3 (10% golden banana puree). The observed variables included pH value, total lactic acid bacteria (LAB) count, and viscosity.

Research Procedure

Development of Banana Puree

Golden banana puree was prepared following the methods of Kartika *et al*. (2020) and Tuhumury *et al*. (2018), with some modifications. Fully ripe golden bananas with yellow skin were sorted, cleaned, and steamed for 5 minutes before peeling. After steaming, the bananas were mashed using a blender to obtain the puree.

Development of Kefir

Kefir was produced by adding banana puree according to the procedure outlined by Mulyani *et al*. (2021) with modifications. One liter of milk was pasteurized for 30 minutes at 65°C. After cooling to room temperature, banana puree was added at the specified treatment levels (6%, 8%, and 10% of milk volume). The mixture was stirred gently, then inoculated with 5% kefir grains aseptically and stirred again. The milk was incubated at room temperature (20-25°C) for 24 hours. After incubation, the kefir was filtered through a 40-mesh nylon sieve to separate the kefir grains (Purba *et al*., 2018).

pH Value Measurement

The pH value was measured using a pH meter. The electrode was cleaned with distilled water and calibrated with pH buffer solutions of 4 and 7 before use. The electrode tip was then inserted into the kefir sample until the pH meter displayed a stable reading, which was recorded (Prastujati *et al*., 2018).

Measurement of Total Lactic Acid Bacteria (LAB)

The total LAB count was determined using the Total Plate Count (TPC) method. Dilutions of 10^-5 and 10^-6 were prepared. One milliliter of each dilution was plated onto sterile deMan Rogosa Sharpe Agar (MRSA) and mixed using the pour plate method. The plates were incubated for 24 hours at 37°C. After incubation, colonies were counted (Sinurat *et al*., 2018).

Viscosity Measurement

Viscosity was measured using the Ostwald pipette method as described by Prastiwi *et al*. (2018). Initially, the specific gravity of the milk was determined by weighing an empty pycnometer, adding 10 ml of the sample, and weighing it again. For viscosity measurement, 10 ml of the sample was placed into an Ostwald pipette. The time taken for the sample to descend from the top to the bottom mark was recorded using a stopwatch. The viscosity was then calculated using the formula:

$$\text{viscositas} = \frac{\rho \text{ x t sample x } \eta \text{ water}}{\rho \text{ x t water}}$$

$$\rho \text{ sample} = \frac{m - m}{v}$$

information :
m : mass of the empty pycnometer (g)
m : mass of pycnometer + sample (g)
v : pycnometer volume (ml)
η : water viscosity
ρ : density (g/ml) (water = 1 g/ml)
t : time (s)

Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) to determine the effects of different treatments on pH value, total lactic acid bacteria (LAB), and viscosity. When significant effects were detected, the Duncan Multiple Range

Test (DMRT) was performed to compare means between treatments. Statistical analysis was conducted using SPSS version 16.0 for Windows with a significance level set at 1%.

RESULTS AND DISCUSSION

The results for pH values, total lactic acid bacteria, and kefir viscosity with the addition of golden banana puree (Musa acuminata) at levels of 0%, 6%, 8%, and 10% are summarized in Table 1.

Table 1. pH value, total lactic acid bacteria and kefir viscosity the with addition banana *puree*

Variable	Treatment			
	P0	P1	P2	P3
pH	4.93±0.15 [c]	4.57±0.06 [b]	4.43±0.06 [b]	4.03±0.06 [a]
Total LAB (CFU/gr)	$1.1x10^4$±0.207 [a]	$8.9x10^4$±0.065 [b]	$1.3x10^5$±0.049 [b]	$2.7x10^7$±0.086 [c]
Viscosity (cp)	8.78±0.95 [a]	12.17±0.76 [ab]	13.63±0.68 [b]	14.93±3.81 [b]

Note: [a, b, c] different lowercase superscript letters indicate very significant differences (P<0.01)

pH Value

The results showed that the pH values for kefir with golden banana puree at different concentrations were as follows: 4.93 for the control (P0), 4.57 for 6% banana puree (P1), 4.43 for 8% banana puree (P2), and 4.03 for 10% banana puree (P3) (Table 1). The analysis of variance revealed that the addition of golden banana puree significantly affected the pH value of kefir (P<0.01). The kefir with 10% banana puree exhibited the lowest pH (4.03), while the control had the highest pH (4.93).

The significant difference in pH values indicates that kefir with 10% golden banana puree is more acidic compared to the control and other treatments. The control treatment had the highest pH value due to the absence of additional carbohydrates. Without extra carbohydrates, the fermentation media had limited nutrients for lactic acid bacteria (LAB) to produce lactic acid, leading to a higher pH (Rizqiati *et al*., 2021).

As the concentration of banana puree increased, the pH value decreased. This is attributed to the carbohydrates present in bananas, which are fermented by LAB into lactic acid. The production of lactic acid during fermentation increases the acidity and lowers the pH value (Utomo *et al*., 2019; Rasbawati *et al*., 2019). Lactose, the primary carbohydrate in milk, is also broken down into lactic acid during fermentation, contributing to the lower pH value as the fermentation progresses (Martharini and Indratiningsih, 2017).

The trend observed in this study aligns with findings from Barus *et al*. (2019), who noted that higher lactic acid production results in a more sour taste and lower pH. The pH values observed in this study are consistent with those reported by Yousefvand et al. (2022), where kefir pH on the first day of fermentation ranged between 4.49 and 4.53. Similarly, Kartika *et al*. (2020) found that increasing concentrations of gembili fruit puree led to a decrease in pH values. The pH of fermented beverages is influenced by organic acids such as lactic acid, acetic acid, butyric acid, and propionic acid produced during fermentation (Viogenta *et al*., 2021).

Total LAB

Lactic acid bacteria are the main microbes used in milk fermentation (Savaiano and Hutkins, 2021). Data on LAB kefir with the addition of golden banana with different levels can be seen in Table 1. The addition of golden bananas at different levels had a very significant effect (P<0.01) on the total LAB of kefir. Table 1 shows that the lowest total LAB was in the control treatment ($2.7x10^7$ CFU/g) and the highest total LAB was in the 10% golden banana treatment ($2.7x10^7$ CFU/g). The higher the golden banana concentration (up to 10%), the greater the LAB growth. This phenomenon occurs allegedly due to the addition of golden bananas that contains glucose and fructose was able to support the development of LAB. In other words, the addition of golden bananas can increase the total activity of lactic acid bacteria so that the number of microbes in kefir increases. According to Meghwar *et al* (2021) bananas contain fructose where it was used as an energy source for the LAB starter so that the growth was optimal during the fermentation process and was able to breaks down lactose into lactic acid. This is in accordance with the statement by (Pratangga, D et al., 2019) that LAB utilizes the sugar in the fermentation media for its growth. The utilization of sugars in the substrate for LAB growth can be seen by increasing the LAB cell population. Sucrose from sugar will be broken down by yeast using the enzyme invertase to produce glucose and fructose, then the results will be utilized by lactic acid bacteria and yeast to produce pyruvic acid through the glycolysis process. The pyruvic acid obtained from the glycolysis process will be converted into lactic acid and will be converted into alcohol and carbon dioxide (Mulyani et al., 2021). Sucrose in bananas and lactose in milk are part of the disaccharides which play an important role for LAB to carry out the fermentation process. LAB growth was influenced by several factors including free water content (a_w), oxygen, availability of substrate in the growth medium, environmental temperature and the initial pathogenic microbes (Pratangga, D et al., 2019).

The results of this study was similar to (Agustine et al., 2018) that yoghurt with additional levels of sucrose and skim milk had increased results, this indicates that the higher variation of sucrose and skim milk will resulting in higher LAB activity due to the presence of sucrose which is a carbon source for the growth of microorganisms.

Viscosity

Lactic acid bacteria (LAB) are crucial for the fermentation of milk, as they produce lactic acid that affects the texture and flavor of kefir (Savaiano and Hutkins, 2021). The data on LAB counts in kefir with varying levels of golden banana puree are presented in Table 1. The addition of golden banana puree significantly influenced the total LAB count (P<0.01). The control treatment (P0) had the lowest LAB count ($2.7x10^7$ CFU/g), while the kefir with 10% golden banana puree (P3) had the highest LAB count ($2.7x10^7$ CFU/g). The increase in LAB count with higher banana puree concentrations can be attributed to the presence of glucose and fructose in bananas, which support LAB growth.

Bananas provide fructose, which serves as an energy source for LAB, thereby enhancing their growth during fermentation (Meghwar *et al.*, 2021). LAB utilize the sugars present in the fermentation medium for growth, which is evident from the increase in LAB counts. Sucrose in bananas and lactose in milk are disaccharides that are broken down during fermentation, aiding in LAB growth and lactic acid production (Mulyani *et al.*, 2021). The presence of sucrose and lactose as carbon sources plays a crucial role in LAB fermentation processes (Pratangga, D *et al.*, 2019). This is similar to findings by Agustine *et al.* (2018), who observed that increasing sucrose and skim milk levels in yogurt led to higher LAB activity.

The viscosity of kefir increased significantly with the addition of golden banana puree (P<0.01). The control treatment (P0) had the lowest viscosity (8.78 cP), while the 10% banana puree treatment (P3) had the highest viscosity (14.93 cP). This increase in viscosity with higher banana puree concentrations can be attributed to the protein content in milk and the effects of banana puree. Proteins in milk bind water, which increases viscosity (Syafitri and Swarastuti, 2013). The presence of golden banana puree enhances the thickening effect as the proteins in milk coagulate due to lactic acid produced during fermentation. This coagulation and thickening are due to proteins reaching their isoelectric point at a pH of 4.7.

Similar findings were reported by Nurubay *et al.* (2021), who observed increased viscosity in goat's milk kefir with added bile banana puree. Paredes *et al.* (2022) also found increased viscosity in kefir with the addition of fruit and vegetable juices. Kefir grains, which contain LAB and yeast, contribute to viscosity through the production of exopolysaccharides (Jurášková *et al.*, 2022). Exopolysaccharides, produced by LAB, enhance the texture and flavor of kefir and provide several health benefits, including wound healing and immunomodulatory effects (Angelin and Kavitha, 2020; Hussain *et al.*, 2017). LAB strains such as Lactococcus, Bifidobacterium, and others are known for producing exopolysaccharides (Sanalibaba and Çakmak, 2016). Additionally, factors such as temperature, incubation time, and the concentration of raw materials affect the viscosity of kefir (Putri *et al.*, 2020).

CONCLUSION

The study concluded that increasing the concentration of golden banana (Musa acuminata) puree in kefir results in higher total lactic acid bacteria (LAB) counts and viscosity, while simultaneously decreasing the pH value.

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