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Optimization of Mangrove Glucomannan Addition to Improve Physicochemical Properties of Kefir

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Abstract. Kefir is widely recognized as a functional fermented dairy product. However, its physicochemical stability, remains a challenge during processing and storage. The addition of functional polysaccharides, such as mangrove-derived glucomannan, has been proposed to improve kefir quality. This study aimed to investigate the effect of mangrove-derived glucomannan on the physicochemical properties of kefir (total bacteria, pH, acidity, and syneresis). A Completely Randomized Design with different concentrations of mangrove glucomannan (0, 2, 4, 6, and 8% (v/v)) was applied, with four replications. The results showed that increasing glucomannan had a significant effect ($P < 0.05$) on total bacteria, pH, and titratable acidity. Mangrove-derived glucomannan significantly enhanced total bacterial counts in kefir ($p < 0.05$), increasing from 6.09 Log CFU/mL in the control to 7.45-7.80 Log CFU/mL at 2-8% concentrations. The treatments decreased pH from 3.66 (0%) to 3.35 (8%) ($P < 0.05$), while titratable acidity increased from 0.70% (0%) to 0.83-0.85% in the treatment groups ($P < 0.05$), confirming enhanced fermentation activity. Syneresis decreased at 2% glucomannan (0.93%) but increased slightly at higher concentrations, reaching 1.03% at 8% ($P > 0.05$). These findings indicate that glucomannan modulates kefir fermentation, as reflected in lower pH and higher total bacteria and acidity.

Keywords: kefir, mangrove glucomannan, prebiotic, pH, titratable acidity, syneresis

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1. Introduction

Kefir is a traditional fermented milk beverage produced through the symbiotic activity of lactic acid bacteria (LAB) and yeast from kefir grains, along with bioactive metabolites that contribute to its probiotic and health-promoting properties [1, 2]. Kefir's nutritional complexity and its potential as a vehicle for delivering functional ingredients support its reported antidiabetic, antioxidant, antihypertensive, anticancer, antimicrobial, and anti-inflammatory effects, as well as its ability to lower cholesterol [3, 4].

One such strategy involves the incorporation of prebiotics, which are non-digestible dietary fibers that selectively stimulate the growth and activity of beneficial microorganisms [5]. When combined with probiotics, these substrates give rise to synbiotic products, offering both microbial and metabolic benefits [6]. In dairy fermentation, prebiotics not only promote LAB viability but also influence important physicochemical attributes, such as acidity, viscosity, water-holding capacity, and overall texture [7]. This matters because improvements in these attributes can enhance both product stability and consumer acceptability [8].

Among various prebiotic sources, plant-derived polysaccharides have received considerable attention [9, 10]. Inulin, fructooligosaccharides (FOS), and resistant starches have been widely applied to dairy-based systems, yielding positive effects on fermentation kinetics and product rheology [11]. However, non-conventional prebiotic sources with unique functional characteristics remain less explored. Glucomannan, a water-soluble polysaccharide known for its high viscosity and gel-forming capacity, represents a promising candidate [12]. While konjac-derived glucomannan has been extensively studied, mangrove-derived glucomannan remains underutilized in food applications, despite its abundance in coastal ecosystems and its favorable physicochemical properties.

The use of prebiotics has attracted considerable attention as a means to improve both the functional and physicochemical characteristics of fermented products [13, 14, 15]. Glucomannan derived from mangroves represents a promising natural prebiotic source, distinguished by its ability to form gels and retain water, which can potentially affect fermentation dynamics, acidity levels, and textural attributes. However, limited studies have explored its utilization in kefir production, particularly how varying concentrations may impact overall product quality. This study, therefore, aims to optimize the level of mangrove-derived glucomannan addition in kefir by examining its physicochemical properties (total bacteria, pH, titratable acidity, and syneresis).

2. Methods

Kefir grains were supplied by a local supplier in West Java. Goat milk was obtained from a farm in Purworejo Regency, Indonesia. *Lactobacillus plantarium* was provided by Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. Mangrove fruits utilized in this study were from *Bruguiera gymnorrhiza*. These mangroves were sourced from the coastal area of Cilacap, Central Java. Mangrove-derived glucomannan was prepared using an extraction method [16].

2.1. Kefir Preparation

Kefir grains were activated into fresh milk at room temperature for 24 h. Milk was pasteurized at 80°C for 15 minutes [17]. Kefir grains were then added to the milk at a ratio of 5%. Goat milk was fermented for 12 h at 28°C. Mangrove-derived glucomannan and *Lactobacillus plantarum* were added at different concentrations (0, 2, 4, 6, and 8% (v/v)) during the fermentation process. After incubation, the mixture was filtered using a sterilized filter to separate the kefir grains. This experiment was repeated four times.

2.2. Total Bacteria

Total bacterial counts were determined using the pour plate method [17]. Kefir samples were serially diluted tenfold in 0.85% NaCl solution. Aliquots (1 mL) from the 10^{-5}, 10^{-6}, and 10^{-7} dilutions were transferred into sterile Petri dishes containing Plate Count Agar (PCA). Incubation was carried out at

37°C under anaerobic conditions for 24 to 48 h. After incubation, plates containing 30-300 colonies were selected. The viable counts were expressed as Log CFU/mL.

2.3. pH Measurement

pH values were measured using a calibrated digital pH meter. The instrument was calibrated with standard buffer solutions at pH 4.00 and pH 7.00 at 25°C, following the manufacturer's instructions. Prior to measurement, approximately 10 g of well-homogenized kefir was transferred into a beaker and equilibrated to 25°C. The electrode was rinsed with distilled water, gently dried with lint-free tissue, and immersed in the sample. Readings were recorded after stabilization, and the electrode was rinsed between samples.

2.4. Titratable Acidity Measurement

Titratable acidity was determined by titration following the procedure in [18] with slight modification. A 10.0 g sample of homogenized kefir was weighed into a 100 mL Erlenmeyer flask. Two drops of phenolphthalein indicator were added, and the sample was titrated with 0.1 N NaOH until a faint pink endpoint persisted. Titratable acidity was calculated as lactic acid equivalents.

2.5. Syneresis Measurement

Syneresis of kefir was determined using a centrifugation-based method. Briefly, 30.0 g of homogenized kefir was transferred into a pre-weighed 50 mL centrifuge tube, and the initial sample weight was recorded. The sample was then centrifuged at 4,000–5,000 rpm for 10 minutes at 4°C. After centrifugation, the separated whey was carefully decanted into a pre-weighed container, and its mass was measured. Syneresis was calculated as the percentage of whey separated relative to the initial sample weight.

2.4. Data Analysis

The kefir data obtained were analyzed statistically using SPSS software. Data were analyzed using one-way ANOVA. When a significant difference was observed, Duncan's Multiple Range Test was performed at a significance level of $p < 0.05$.

3. **Results and Discussion**

3.1. Total Bacteria

The total bacteria of symbiotic kefir increased significantly with the addition of mangrove-derived glucomannan ($p < 0.05$). As shown in Figure 1. kefir without glucomannan (0%) exhibited the lowest bacterial count (6.09 Log CFU/mL). Kefir treated with glucomannan at concentrations of 2, 4, 6, and 8% showed significantly higher bacterial counts, ranging from 7.45 to 7.80 Log CFU/mL. This increase may be attributed to the presence of dietary fibers in mangrove glucomannan, which act as prebiotic compounds that stimulate probiotic growth.

The addition of mangrove-derived glucomannan influenced total bacterial counts, indicating an active fermentation process in the presence of this polysaccharide. A plausible explanation is that mangrove glucomannan supports symbiotic interactions among microbial cultures in kefir. [19] reported that the inulin content in lesser yam flour can support the symbiosis of three bacterial cultures in yogurt, allowing LAB to grow optimally. Similar findings have been reported in fermented dairy products supplemented with dietary fibers, where fermentable substrates promote organic acid production during extended fermentation [20]. Previous studies also showed that kefir supplemented with Jerusalem artichoke prebiotics achieved LAB counts above 8 Log CFU/mL, indicating that prebiotics can effectively support LAB growth.

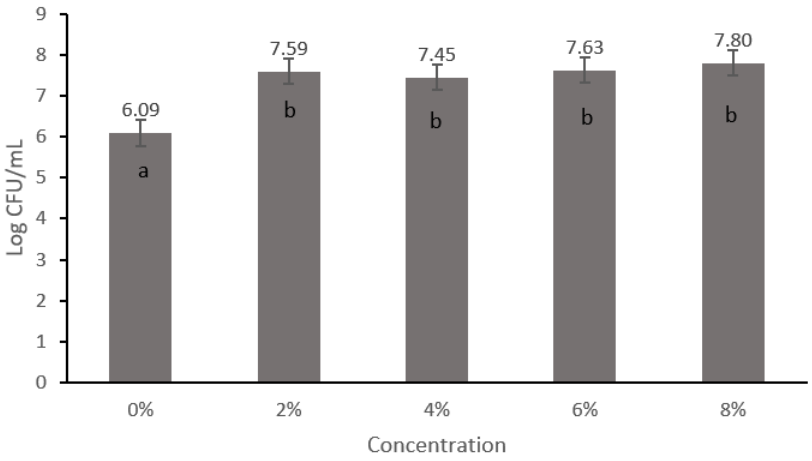

Figure 1. Total Bacteria of Kefir

3.2. pH Value

The addition of mangrove-derived glucomannan significantly affected the pH of kefir (Figure 2). The pH value differed significantly with glucomannan addition ($P < 0.05$). The control (0%) exhibited the highest pH value (3.66), while the lowest pH was observed at 8% glucomannan (3.35). Increasing glucomannan concentration tended to decrease pH, indicating enhanced acidification during fermentation. This may be attributed to the role of glucomannan as a prebiotic substrate, which stimulates LAB metabolism and organic acid production. As a soluble dietary fiber, glucomannan may improve the fermentation microenvironment by enhancing water availability and matrix structure, thereby supporting microbial activity.

A decrease in kefir viscosity may occur due to cytoplasmic glycohydrolase activity after a certain incubation period, which can degrade exopolysaccharides [21]. [22] reported that kefir produced from *Aronia melanocarpa* juice and pomace showed a decrease in pH value. Similar findings have been reported, where prebiotic fibers promoted microbial activity, leading to reduced pH in fermented kefir products [22, 23, 24]. The pH also plays a role in molecular interactions and transport properties [25].

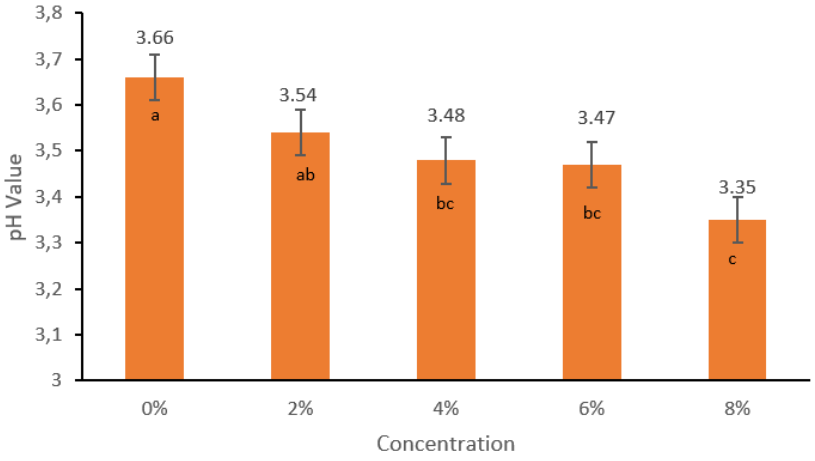

Figure 2. pH Value of Kefir

3.3. Titratable Acidity (TA)

The titratable acidity (TA) of kefir is presented in Figure 3. TA increased from 0.70% at 0% glucomannan to 0.83-0.85% at higher concentrations. This increase confirms enhanced fermentation, consistent with the observed decrease in pH. The statistical grouping shows that kefir with glucomannan addition (2-8%) had significantly higher TA than the control ($P < 0.05$). One plausible explanation is that lactic acid fermentation converts carbohydrates into organic acids, such as lactic acid and acetic acid. This suggests that even low levels of glucomannan are sufficient to support LAB activity and sustain acid production. Previous studies have reported similar findings, where prebiotics and dietary fibers stimulate lactic acid formation in kefir [26, 27].

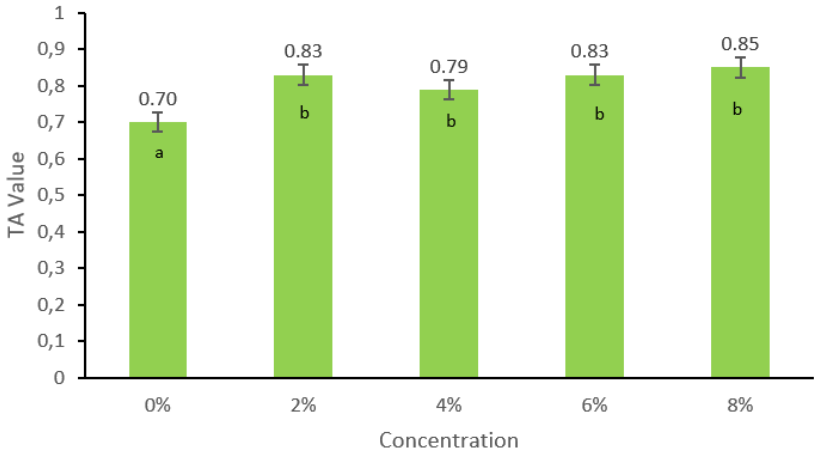

Figure 3. Titratable Acidity Value

3.4. Syneresis Value

Syneresis influences the consistency and appearance of the final kefir product. Syneresis The values ranged from 0.93% to 1.03% (Figure 4). The addition of mangrove-derived glucomannan showed no statistically significant difference in kefir syneresis ($P > 0.05$). The lowest syneresis was recorded at 2% glucomannan (0.93%), while the highest was observed at 8% (1.03%). A slight reduction in syneresis at low concentrations may be explained by the high water-holding capacity of glucomannan, which helps improve the gel matrix and reduce whey separation. However, at higher concentrations (6-8%), syneresis tended to increase, possibly because excessive fiber interferes with protein-gel interactions, leading to structural instability. This finding is consistent with previous literature showing that the use of apricot (*Prunus armeniaca* L.) seed extract in kefir production did not improve syneresis values [22]. Other studies have reported that moderate levels of hydrocolloids can enhance texture and reduce whey loss, while excessive addition may destabilize the matrix [23].

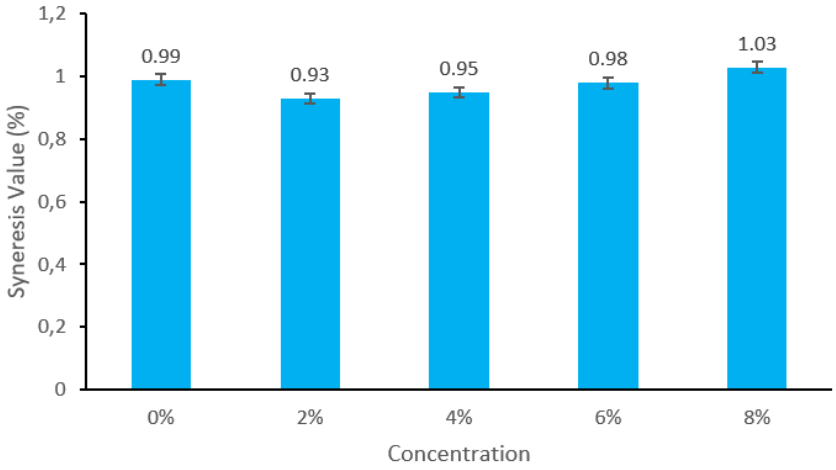

Figure 4. Syneresis Value

4. Conclusion

In conclusion, the addition of mangrove-derived glucomannan significantly influenced total bacteria and the physicochemical properties of kefir. Increasing glucomannan concentrations enhanced acidification, as reflected in decreased pH and increased titratable acidity. The addition of glucomannan at concentrations of 2-4% reduced syneresis values. These results indicate that mangrove-derived glucomannan can be used to improve the physicochemical properties of kefir, particularly total bacteria, pH, and acidity.

Declaration of AI and AI assisted technologies in the writing process
During the preparation of this work the author used ChatGPT in order to guarantee that the choices of words. After using this tool/service, the author reviewed and edited the content as needed and take full responsibility for the content of the publication

Declaration of Competing Interest
The authors declare that there have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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