The Addition of Acacia Honey to Kefir Results in Changes to Physicochemical And Microbiological Properties

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ABSTRACT

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| This study aimed to evaluate the impact of adding acacia honey on the physicochemical and microbiological properties of cow's milk kefir. The research was conducted from February to April 2025 at the Laboratory of Dairy Science and Technology at Universitas Brawijaya, employing a five-treatment experimental design with three replications: 0%, 2%, 4%, 6% and 8% honey concentrations. The milk was pasteurised at 70 °C, cooled to 40 °C, mixed with honey and inoculated with 5% kefir grains by weight. The mixture was then fermented for 24 hours at 27–28 °C. Increasing the honey concentration was found to slightly elevate the pH level (from 3.88 to 3.97), while significantly reducing the viscosity level (from 7.44 to 1.99 Pas). Total titratable acidity (TTA) increased at a honey concentration of 2% (1.94%), but decreased at higher concentrations. Microbiological analysis revealed that adding honey increased both lactic acid bacteria (LAB) and yeast populations to 6.89 and 6.08 log CFU/mL, respectively, at an 8% honey concentration. These values are close to the minimum threshold for probiotic functionality (≥7 log CFU/mL), suggesting that the product remains within an acceptable range for health benefits. While this study did not examine honey concentrations above 8% in detail, preliminary observations suggested that higher concentrations tend to disrupt the balance of microbes and negatively impact the texture and fermentation process. This results in excessive viscosity and unclear microbial interactions. Therefore, concentrations above 8% are not recommended without further investigation. These results suggest that acacia honey enhances microbial viability and modulates the fermentation process, thereby influencing the acidification and texture of the final kefir product. Consequently, acacia honey can be considered a functional ingredient for tailoring the nutritional and sensory qualities of kefir. |

*Keywords: kefir, acacia honey, physicochemical properties, lactic acid bacteria, viscosity, fermentation*

1. INTRODUCTION

Kefir is a fermented beverage made from milk that is fermented by a consortium of microorganisms, including lactic acid bacteria and yeast (Leite et al., 2013). This combination of microbes results in a product with a distinctive sour flavor and various health benefits, including antimicrobial and probiotic properties (Rosa et al., 2017). Additionally, kefir's composition is strongly influenced by the type of milk used, the fermentation temperature, and the incubation time (Zheng et al., 2020). These factors affect kefir's physicochemical properties, including pH, total acidity, and viscosity (Fiorda et al., 2017).

As a fermented product, kefir has great potential for development through the addition of functional ingredients, such as honey, to improve sensory quality and health benefits (Gül et al., 2015). Honey contains natural sugars, organic acids, enzymes, and bioactive compounds, such as flavonoids and phenolic compounds, which have antimicrobial and antioxidant properties (da Silva et al., 2016). These components affect not only the nutritional profile but also support the growth of probiotic microorganisms during fermentation (Ahmed et al., 2022). Adding honey to kefir has also been reported to alter the final product's physicochemical characteristics by increasing total soluble solids and decreasing pH (Gül et al., 2018).

The type of honey used in fermentation plays an important role because its chemical composition varies depending on the nectar source. One type of honey is acacia honey, which is known for its high sugar content and light color (Alvarez-Suarez et al., 2010). Acacia honey's high fructose content can serve as an energy source for the lactic acid bacteria and yeast in kefir (Zhu et al., 2021). Additionally, acacia honey's phenolic compounds can provide antioxidant effects, enriching kefir's functional value (Yilmaz & Karaaslan, 2020). Given these properties, acacia honey is expected to affect the microbiological profile and physicochemical parameters of kefir during fermentation (Nowak et al., 2021).

While studies have evaluated the effect of honey on the fermentation process of dairy products, studies specifically examining the effect of adding acacia honey to kefir are limited (Gul et al., 2018). In fact, variations in the chemical and bioactive characteristics of certain types of honey, such as acacia honey, have the potential to produce different effects on the microbiological dynamics and physicochemical parameters of kefir (Ahmed et al., 2022). Therefore, this study aimed to evaluate the effect of acacia honey addition on the physicochemical properties (such as pH, viscosity and total acid) and microbiological characteristics (total number of lactic acid bacteria and yeast) of cow's milk kefir.

This research makes a valuable contribution to our scientific understanding of how natural functional ingredients, such as acacia honey, can affect the physical, chemical and microbiological properties of fermented dairy products like kefir. By systematically evaluating the effects of varying honey concentrations, the study provides insights into optimising texture, acidity, microbial and viability factors that are crucial to both consumer acceptance and probiotic efficacy. The findings expand current knowledge on functional food development and support the formulation of bespoke fermented beverages with enhanced health benefits. In light of the growing demand for natural and bioactive food additives, this research addresses technological and nutritional gaps in functional dairy innovation.

1. material and methods
   1. **Materials**

Fresh cow milk from a local dairy supplier in Malang was used as the base for kefir production. To ensure authenticity, acacia honey was sourced from a certified local beekeeper. Commercial kefir grains containing a symbiotic consortium of lactic acid bacteria and yeasts were used as a starter culture.

**2.2. Sample Preparation**

First, the cow milk was pasteurized at 70°C for 10 minutes using the method described by Fiorda et al. (2017). Then, it was allowed to cool down to 40°C. After cooling, acacia honey was added to separate batches at concentrations of 2%, 4%, 6%, and 8% (v/v). Each mixture was manually homogenized for two to three minutes to ensure the honey was evenly distributed. Then, kefir grains were added at 5% (w/v) of the milk volume, after which the mixture was incubated in sterilized glass jars at 27–28 °C for 24 hours under aerobic conditions.

**2.3. Experimental Design**

The research employed a completely randomized design (CRD) with four honey concentration levels (2%, 4%, 6%, and 8%) and three replications per level. Control samples without honey were also prepared for comparison. All samples were processed under the same environmental condition. All collected data were analyzed using a one-way analysis of variance (ANOVA) to determine significant differences among the treatments. If significant differences were found (p < 0.05), a post analysis using Duncan’s Multiple Range Test (DMRT) was performed to identify specific differences between treatments.

**2.4 Physicochemical Analysis**

The pH of the samples was measured using a calibrated digital pH meter (Horiba LAQUAtwin, Japan). Total titratable acidity (TTA) was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator and was expressed as a percentage of lactic acid (AOAC, 2005). Total soluble solids (TSS) were analyzed using a handheld digital refractometer (Atago PAL-1, Japan), and the results were expressed in °Brix. Viscosity was measured using a digital viscometer (Brookfield DV-E, USA) equipped with spindle No. 3 at 100 rpm, and the results were expressed in centipoise (cP), following the procedure described by Prado et al*.* (2015).

**2.5 Microbiological Analysis**

The microbiological analysis included counting lactic acid bacteria (LAB) and yeast using the pour plate method. Serial dilutions of kefir samples were plated on MRS (De Man, Rogosa, and Sharpe) agar for LAB and on PDA (potato dextrose agar) for yeasts. The plates were then incubated at 37°C for 48 hours for the LAB and at 30°C for 72 hours for the yeasts, following the method described by Leite et al. (2013). Colony-forming units (CFUs) were counted and expressed as log CFUs/mL.

1. results and discussion
   1. **Physicochemical Characteristic of Kefir**

**Table 1. Physicochemical quality of kefir with added different concentration of acacia honey**

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| **Type of Product** | **By-product Formulation** | **Physical Quality of Kefir** | **Effect of Addition** | **Reference** |
| Kefir | Control (kefir without added honey) | pH: 3.88  Viscosity: 7.44 Pa  TTA: 1,62% | Kefir without added honey shows active fermentation with high acidity and a thick texture. | Fiorda et al. (2017) |
| Kefir | Addition of 2% acacia honey | pH: 3.94  Viscosity: 4.88 Pa  TTA: 1.94% | The addition of 2% honey to kefir showed an increase in acidity and a decrease in viscosity due to the contribution of sugar and bioactive compounds from honey to fermentation activity. | Gul et al. (2015) |
| Kefir | Addition of 4% acacia honey | pH: 3.95  Viscosity: 2.63 Pa  TTA: 1.67% | Kefir with 4% honey added showed that increasing the honey concentration accelerated acid metabolism but also reduced viscosity due to dilution of the kefir gel structure. | Nowak et al. (2021) |
| Kefir | Addition of 6% acacia honey | pH: 3.92  Viscosity: 3.78 Pa  TTA: 1.73% | The addition of 6% honey to kefir showed a balance between increased acid production and improved viscosity, possibly due to the interaction of prebiotic compounds in honey with microbial activity and protein matrix formation. | Oliveira et al. (2021) |
| Kefir | Addition of 8% acacia honey | pH: 3.97  Viscosity: 1.99 Pa  TTA: 1.36% | Kefir fermented with the addition of 8% honey showed a decrease in fermentation activity and physical structure due to the possible osmotic effect of high honey concentration on microbes and product texture. | da Silva et al. (2016) |

The pH values of the kefir samples varied depending on the concentration of acacia honey added during fermentation. The control sample, which did not contain honey, had the lowest pH value of 3.88, reflecting high acidity due to normal lactic acid production during fermentation. Adding 2% or 4% honey slightly increased the pH values to 3.94 and 3.95, respectively. This suggests that the additional sugars in the honey may have buffered the acid production or altered the microbial metabolism. This buffering effect has been observed in fermented dairy systems with carbohydrate-rich ingredients. Available sugars reduce stress for fermentative microbes, resulting in moderated acid release (Sung et al., 2019).

Interestingly, kefir with 6% and 8% honey had even higher pH values (3.92 and 3.97, respectively), indicating a trend of reduced acid accumulation at higher honey concentrations. This may be due to the effects of osmotic pressure caused by high sugar levels, which can inhibit excessive microbial acidification. Additionally, honey components such as flavonoids and phenolic acids have antioxidant and antimicrobial properties that can modulate microbial growth and metabolic activity, thereby affecting the product's pH (Ahmed et al., 2020). Taken together, these findings suggest that acacia honey serves as a fermentable substrate and alters the acidification dynamics of kefir during fermentation.

The viscosity of kefir decreased progressively as the concentration of acacia honey increased, indicating that the addition of honey plays a crucial role in modulating the product’s texture. The control sample, which did not contain honey, had the highest viscosity at 7.44 Pas. This is typical of traditionally fermented kefir, which forms a stable gel matrix. When 2% or 4% honey was added, the viscosity dropped sharply to 4.88 Pas and 2.63 Pas, respectively. This decline may be due to the dilution of the protein network from the added liquid sugar and honey components interfering with casein micelle interactions that stabilize the kefir matrix (Hashemi & Jafarpour, 2016).

At higher honey concentrations (6% and 8%), viscosity decreased further to 3.78 Pas and 1.99 Pas, respectively. This continuous reduction in viscosity, despite good fermentation activity, suggests that high sugar concentrations may disrupt exopolysaccharide production or limit gel strength by affecting water-holding capacity. Additionally, the osmotic effect of concentrated sugars in honey may weaken the kefir's microstructure by reducing protein-protein or protein-polysaccharide bonding. Similar observations were reported by Păcularu-Burada et al. (2022), who found that adding sweeteners to fermented milk altered its rheological behavior due to changes in protein-carbohydrate interactions and moisture distribution.

The total titratable acidity (TTA) of kefir changed with the concentration of added acacia honey, indicating alterations in lactic acid production during fermentation. The control sample had a TTA of 1.62%, which is typical of the acid produced by lactic acid bacteria in dairy fermentation. Interestingly, adding 2% honey increased the TTA to 1.94%, suggesting that the added sugars enhanced microbial activity and lactic acid synthesis. This finding is consistent with studies demonstrating that simple sugars in natural sweeteners can serve as fermentable substrates, thereby increasing organic acid output (Chowdhury et al., 2020).

However, as the honey concentration increased further (to 4%, 6%, and 8%), the TTA gradually decreased to 1.67%, 1.73%, and 1.36%, respectively. This trend suggests that, while low honey concentrations stimulate fermentation, higher concentrations may suppress excessive acid production due to osmotic stress or the inhibitory effects of certain honey components. Phenolic compounds and the high sugar content of honey have been shown to affect microbial metabolism by altering membrane transport and enzyme function. This could reduce total acid accumulation (Kek et al., 2017). These findings suggest that honey plays a dual role: enhancing microbial activity at lower levels while modulating acidification at higher concentrations.

* 1. **Microbiological Characteristic of Kefir**

**Table 2. Physical quality of kefir with added different concentration of acacia honey**

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| **Type of Product** | **By-product Formulation** | **Physical Quality of Kefir** | **Effect of Addition** | **Reference** |
| Kefir | Control (kefir without added honey) | LAB: 5.85 log CFU/ml  Yeast: 4.97 log CFU/ml | Kefir without honey addition showed stable microbial fermentation activity even without additional carbohydrate source. | Leite et al. (2013) |
| Kefir | Addition of 2% acacia honey | LAB: 6.26  Yeast: 5.67 | Kefir with 2% honey added showed an increase in the number of LAB and yeast, indicating that honey acts as an additional energy source that supports the growth of fermentative microbes. | Medrano et al. (2009) |
| Kefir | Addition of 4% acacia honey | LAB: 6.61  Yeast: 5.80 | Kefir with 4% honey added showed an increase in the number of LAB and yeast, indicating that this concentration of honey optimally supports microbial activity during fermentation. | Rodrigues et al. (2005) and Sanz. (2009) |
| Kefir | Addition of 6% acacia honey | LAB: 6.77  Yeast: 5.97 | Kefir with 6% honey added showed that increasing honey concentration significantly supported the growth of probiotic microbes during fermentation. | Santos et al. (2019) |
| Kefir | Addition of 8% acacia honey | LAB: 6.89  Yeast: 6.08 | Kefir with the addition of 8% honey showed that high honey concentration was able to maximally increase fermentative microbial activity. | Chen et al. (2016) |

Adding acacia honey to kefir significantly affected the population of lactic acid bacteria (LAB). The control sample, which did not contain honey, showed an LAB count of 5.85 log CFU/mL, indicating baseline microbial activity during standard fermentation. However, adding 2% honey increased the LAB count to 6.26 log CFU/mL, suggesting that the sugars and nutrients in the honey acted as an additional energy source for LAB growth. This trend continued with higher honey concentrations; the LAB count reached 6.61 log CFU/mL with 4% honey and 6.77 log CFU/mL with 6% honey. These results align with prior research indicating that natural sugars, including glucose and fructose, present in honey can stimulate the growth of lactic acid bacteria in fermented dairy products (Wang et al., 2018; Haliniak et al., 2020).

Interestingly, the highest LAB count was observed with an 8% honey addition, reaching 6.89 log CFU/mL. This suggests that, in addition to providing fermentable carbohydrates, acacia honey at higher concentrations may also supply minor bioactive compounds, such as phenolics and oligosaccharides, that could enhance microbial growth synergistically. However, excessive sugar could lead to osmotic stress beyond optimal levels. Thus, the observed increase at 8% honey may indicate a threshold concentration that still favors microbial proliferation. Similar results were reported by Al-Mamoori et al. (2021), who found that moderate to high concentrations of honey promoted probiotic strain activity in fermented milk. Furthermore, Rafiee et al. (2022) highlighted that honey supplementation in dairy fermentation can support the viability of lactic acid bacteria without negatively impacting fermentation dynamics.

Adding acacia honey also affected the yeast population in kefir. In the control treatment (without honey), the yeast count was 4.97 log CFU/mL, reflecting baseline fermentation activity under standard nutrient conditions. Adding 2% honey increased the yeast count to 5.67 log CFU/mL, suggesting that the honey provided fermentable sugars, such as glucose and fructose, that supported yeast metabolism. This trend continued with the addition of 4% and 6% honey, reaching 5.80 and 5.97 log CFU/mL, respectively. These results align with studies indicating that moderate levels of natural sweeteners enhance yeast viability by improving substrate availability during co-fermentation with bacteria (Zomorodi et al., 2019; Oliveira et al., 2021).

The highest yeast count (6.08 log CFU/mL) occurred at an 8% honey concentration. This suggests that higher honey concentrations may promote optimal yeast proliferation in the kefir matrix. This could be attributed to the carbohydrate content of honey and the presence of micronutrients and trace elements, such as potassium and magnesium, that support yeast growth. However, excessive sugar can alter osmotic pressure. In this case, though, the yeast appeared to thrive, likely due to the natural compatibility between the honey composition and yeast metabolic pathways. Similar results were reported by Mohammadi et al. (2020), who found that yeast growth in fermented dairy products increased significantly with the addition of functional carbohydrate sources. Additionally, Zhang et al. (2017) observed that yeast populations in kefir-like fermented products are highly responsive to natural prebiotic supplementation, particularly from plant-based sources such as honey.

4. Conclusion

This study demonstrated that the addition of acacia honey at varying concentrations influenced the physicochemical and microbiological properties of kefir. The pH values increased slightly with higher honey concentrations, indicating a buffering or modulating effect on acid production during fermentation. Viscosity decreased progressively as honey concentration increased, suggesting structural weakening of the kefir matrix likely due to the osmotic and diluting effects of sugars. Total titratable acidity peaked at moderate honey addition (2%) but declined at higher levels (8%), reflecting the dual role of honey in enhancing and regulating fermentation activity. Furthermore, microbial analysis revealed that both lactic acid bacteria and yeast populations increased with honey supplementation, with the highest counts observed at 8%, confirming the role of honey as a fermentable and bioactive carbon source. These findings suggest that acacia honey can be used strategically to modify kefir quality, depending on the desired texture, acidity, and microbial profile of the final product.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as large language models (chatgpt, copilot, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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