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Research Article

Comparative Analysis of Nutritional and Microbial Profiles of Kefir from Diverse Regions in Indonesia

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Abstract | Kefir is a probiotic fermented milk product known with various health benefits, including improving digestion, supporting the immune system, having potential of anti-inflammatory properties, antioxidant activity, and its ability to inhibit the alpha-glucosidase enzyme that can help regulate blood sugar levels. However, the availability of kefir in Indonesia still needs to be improved. Kefir grains developed in Indonesia come from countries such as Malaysia, Turkey, Brazil, Russia, Argentina, and China. These grains are then cultivated in six different regions in Indonesia. This study aimed to conduct a comparative analysis of kefir grains developed from six regions in Indonesia: East Java (KG1), North Kalimantan (KG2), West Java (KG3), Central Java (KG4), North Sumatra (KG5), and West Sumatra (KG6). This study used an experimental method with a Completely Randomized Design (CRD) consisting of six treatments with five replications. The analysis focused on physicochemical characteristics (water content, protein, ash, lactose, fat, viscosity, total phenolics, pH, and total titration acidity), microbiological properties (number of lactic acid bacteria and yeast), antioxidant activity, and inhibition of α-glucosidase enzyme. Physicochemical characteristics showed significant differences except for protein content. Total phenolic content, antioxidant activity, and inhibition of α-glucosidase enzyme also varied among kefir fermented from various grain sources. Total lactic acid bacteria and yeast in kefir showed significant differences among the area of kefir grains origin. The best kefir quality was obtained from kefir grain starters from North Sumatra (KG5) based on the comparison of the regions Overall, kefir grain starters developed in 6 regions in Indonesia had shown the ability to produce good quality kefir.

Keywords | Antioxidant, α-Glucosidase inhibition, Kefir grains, Lactic acid bacteria, Yeast

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INTRODUCTION

Kefir, a traditional fermented dairy product, is globally valued for its probiotic properties and health benefits. Its growing demand as a functional food is driven by consumer interest in gut health and immunity. Kefir originally comes from the Caucasus Mountains (Russia) but has been known and spread to various parts of the world. Kefir grains cultivated in Indonesia originate from Malaysia, Turkey, Brazil, Russia, Argentina and China. These kefir grains are then developed in 6 different regions in Indonesia, namely West Sumatra, North Sumatra, North Kalimantan, West Java, Central Java and East Java. Generally, the production and development of kefir in Indonesia still need improvement. The Indonesian Kefir Community (IKC) has been established to unite Kefir activists throughout the country (Yusuf *et al*., 2021). This organization routinely and continuously provides training and guidance to kefir producers. These producers produce kefir daily, although still on a home industry scale. Kefir products are generally marketed within the IKC community and to some members of the general public looking for the health benefits of consuming kefir.

The kefir was fermenting milk by a kefir grain starter composed of a symbiotic complex of approximately 300 microbial species, including lactic acid bacteria, acetic acid bacteria and yeasts. The main groups of microorganisms present in kefir grains consist of Lactobacillus, homofermentative mesophilic Lactococci, heterofermentative mesophilic Lactococci, lactose-fermenting and non-lactose-fermenting yeasts, Filamentous fungi, and Acetic acid bacteria (Rosa *et al*., 2017). The yeast population in both kefir grains and kefir itself can vary. Common yeasts found in kefir grains consisted of *Saccharomyces* sp., *Kluyveromyces lactis*, *Kazachstania* sp., and *Candida* sp. (Marsh *et al*., 2013). Physically, kefir grains resemble small cauliflower florets with the diameters ranging from 0.3 to 1.3 cm. They have irregular shape with vaired surfaces that form folded to smooth, and they are white to yellowish-white in color with a slimy texture. When pressed, kefir grains are elastic (Van Wyk, 2019). Kefir grains comprise approximately 83% water, 4-5% protein, and 9-10% polysaccharides (kefiran) (Bengoa *et al*., 2018).

The physicochemical composition of kefir grains can vary depending on their source. These differences may depend on the diversity of milk sources and the duration of kefir grain cultivation. Additionally, sanitation practices during the handling of kefir grains may lead to variations in the microflora composition of the grains (Vedamuthu, 2013). The quantity of microflora in kefir grains may change over time because of the storage, maintenance, and reuse of the grains. Furthermore, varying temperature conditions may influence the diversity and abundance of microflora in kefir grains. Kefir offers various health benefits, including antiviral and anticancer properties and the potential to prevent degenerative diseases (Ferawati *et al*., 2022). Further research is needed to fully understand the role of kefir in reducing blood sugar levels. The decrease in blood sugar may be attributed to several bioactive compounds in kefir, including exopolysaccharides, antioxidants, peptides, and immune-boosting properties (Moradi and Kalanpour, 2019). Therefore, kefir plays a significant role in the therapy for managing diabetes. Nonetheless, additional research is essential to provide conclusive evidence regarding these effects.

Despite extensive research, the impact of regional variations in kefir grains on its nutritional and functional properties remains unclear. This study addresses this gap by analyzing kefir from six Indonesian regions to assess geographic influences on its bioactive compounds and probiotic activity. Based on the background above, comparative research on kefir is necessary, focusing on its physicochemical properties, microbiological characteristics, antioxidant activity, and inhibition of α-glucosidase enzyme activity. Kefir grain samples in this study were obtained from six different regions in Indonesia with climate variations, especially in terms of temperature, altitude above sea level, and relative humidity levels. This study utilized kefir grains from household-scale producers in various regions (Table 1).

Table 1: Meteorological data of kefir grain sampling region.

Kefir grain sampling region	Temperature	Relative Humidity	Height above sea level
East Java	21-34°C	±76 %	30 MASL
North Kalimantan	22-34.9°C	±83.59%	24 MASL
West Java	18-34°C	±89%	340 MASL
Central Java	20-30°C	±62%	4 MASL
North Sumatra	23-33.1°C	±79%	37.5 MASL
West Sumatra	21-32°C	±85%	514 MASL

Data Source: Meteorological, climatological, and geophysical agency, Indonesia (2024). **Note:** MASL (meter above sea level).

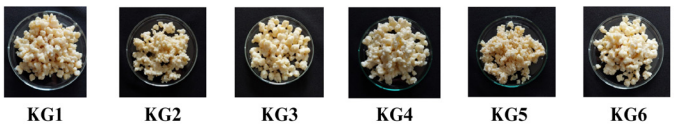

Figure 1: Photographs of kefir grains from different regions.

MATERIALS AND METHODS

Preparation and Activation of Kefir Grains

This study selected six locations for producing kefir grains due to differences in the country of origin of the kefir grains (Figure 1). This kefir grain has been developed for at

least 5 years in various regions in Indonesia, previously kefir grains came from different countries, namely East Java kefir grains from Russia (KG1), North Kalimantan kefir grains from Malaysia (KG2), West Java kefir grains from Argentina (KG3), Central Java kefir grains from Turkey (KG4), North Sumatra kefir grains from China (KG5) and West Sumatra kefir grains from Brazil (KG6). Kefir grains were stored at 4°C before activation. Prior to use, they were activated through successive fermentations in fresh goat milk at 25°C for 24 hours, with the fermentation medium replaced every 24 hours to ensure full adaptation. This activation phase was essential for stabilizing microbial activity and optimizing kefir fermentation. In this study, each kefir grain originating from different sources was separately weighed as much as 250 grams and mixed with 2500 mL of pasteurized Etawa goat milk (10% w/v). The choice of pasteurized goat milk from traditional dairy farm in Payakumbuh city, known for its high nutritional value with the following nutritional content: total fat 4.8%, protein 3.5%, lactose 4.1%, and low microbial load, was made to ensure a consistent and high-quality fermentation process. Fermentation was conducted at 37°C for 24 hours. After fermentation, the kefir grains were strained using a 100-mesh stainless steel sieve to separate them from the kefir. The kefir was matured for 12 hours at refrigerator temperature (5-8°C) (Ferawati *et al*., 2024). Kefir was ready to be analysed.

Experimental Design

This study employed an experimental method using a Completely Randomized Design (CRD) with six treatments and five replications. The treatments consisted of kefir grains sourced from different regions (KG1, KG2, KG3, KG4, KG5, and KG6). The objective of the research was to determine the physicochemical characteristics of kefir, including moisture, ash, protein, lactose, fat, viscosity, pH, and Total Titratable Acidity (TTA). Additionally, the study evaluated the total phenolic content, free radical scavenging activity, inhibition of the α-glucosidase enzyme, and microbiological properties, specifically the colony counts of lactic acid bacteria and yeast.

Proximate Analysis

The kefir samples were analyzed for moisture, ash, protein, fat, and lactose content following the methods outlined in ISO (2014). The pH of the samples was measured using a pH meter (HANNA) that was calibrated with buffer solutions of known pH values (7, 4, and 10). The pH meter probe was immersed into a 5 mL pyrex beaker containing 3 mL of kefir sample, and the pH values were recorded accordingly (Sulmiyati *et al*., 2019). The total titratable acidity (TTA) was determined according to the method described by Purnomo and Muslimin (2012). Acid content was measured by titrating 5 mL of kefir with 0.1 N NaOH, using two drops of phenolphthalein indicator (PP Indicator), until the sample turned pink. Acid levels calculation was performed as follows:

$$Total\ titratable\ acidity = \frac{(mL\ NaOH\ \times\ 0.009)}{(weight\ of\ milk\ (g)) - 1} \times 100\%$$

Determination of Kefir Viscosity

The measurement of kefir viscosity followed the methodology outlined by Ahmed *et al*. (2013). This step was started by placing 250 mL of kefir into a 500 mL beaker, ensuring that the sample was maintained at a constant temperature, typically within the range of 20-25°C (room temperature). Calibrate the Brookfield viscometer according to the manufacturer's instructions, utilizing viscosity calibration standards to ensure measurement accuracy. For kefir analysis, employ either spindle LV2 or LV3, as the viscosity of kefir generally falls within the low to medium range. Set the rotation speed, typically between 50-60 rpm, for viscosity measurement. Submerge the spindle into the kefir sample to the appropriate depth, ensuring that it does not contact the bottom or sides of the beaker. Allow the spindle to rotate for 1-2 minutes until a stable reading is achieved. The measured viscosity was displayed in centipoise (cP). Record the viscosity value as indicated by the viscometer.

Microbiological Analysis

The growing colonies of lactic acid bacteria were determined using the method (Tomar *et al*., 2018). The first dilution series was 1 mL of kefir sample added to 9 mL of sterile de Man Rogosa Sharpe broth (MRS-broth, Merck) with dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} then vortexed (Schoot). Dilutions 10^{-5} and 10^{-6} were carried out with 0.1 mL de Man Rogosa Sharpe Agar (MRS-Agar, Merck) into a petri dish. We used a temperature of 37°C for 48 hours during incubation, observe the growing colonies, and count them using a colony counter (WTW, BZG 30). Yeasts were cultured on potato dextrose agar (Merck, 1.10130) (pH 3.5) with 10% added tartaric acid (Akarca *et al*., 2016). The calculation was determined by selecting colonies that grew from 25-250 colonies in a petri dish and included as follows:

$$Colony/gram\ (CFU/mL) = The\ number\ of\ colonies \times \frac{1}{dilution\ factor}$$

Determination of α-Glucosidase Enzyme Inhibition Activity

The α-glucosidase inhibitory activity was determined using spectrophotometric analysis, employing p-nitrophenyl-α-D-glucopyranoside (pNPG) as a substrate. The inhibition activity of α-glucosidase was evaluated according to the method described by Setiawan *et al*. (2024). The reaction mixture consisted of 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of 4-nitrophenyl-α-D-glucopyranoside (dissolved in 0.1 M phosphate buffer, pH 7.0), 10

µL of the sample (10 mg dissolved in 1 mL of dimethyl sulfoxide and distilled water), and 25 µL of the α-glucosidase enzyme solution (0.04 units mL^{-1} in 0.1 M phosphate buffer, pH 7.0). The mixture was incubated at 37°C for 30 minutes. After incubation, the reaction was terminated by adding 100 µL of 0.2 M sodium carbonate solution. The extent of enzymatic hydrolysis of the substrate was assessed by quantifying the amount of p-nitrophenol released during the reaction. This measurement was performed using a microplate reader at a wavelength of 410 nm.

Determination of Total Phenolic Content and Free Radical Scavenging Activity

Samples were prepared following the method described by Goraya and Bajwa (2015). Two grams of the sample were dissolved in 25 mL of 80% methanol solution and left to extract for 1 hour. The mixture was then filtered using Whatman filter paper no. 1 and diluted with methanol to a final volume of 100 mL. The phenolic content was evaluated using the Folin-Ciocalteu reagent. A 0.1 mL aliquot of the sample extract was mixed with 1 mL of Folin-Ciocalteu reagent (diluted in a 1:9 ratio with distilled water), allowed to stand for 5 minutes and followed by the addition of 1 mL of sodium carbonate, and the volume was increased to 10 mL with distilled water. The solution was maintained at room temperature for 90 minutes before being analyzed using a spectrophotometer at 725 nm. Gallic acid was used as the standard, and the results were expressed in milligrams of gallic acid equivalents (GAE) per gram of sample. The DPPH method, as described by Caleja *et al.* (2016), was employed using the methanol extract to determine the percentage of free radical scavenging activity. The absorbance of the mixture was measured at 515 nm using a spectrophotometer (Model 20D UV, Milton Roy Company, USA), with a DPPH solution without extract serving as the blank. Free radical scavenging activity was calculated as follows:

$$DPPH\ radical\text{-}scavenging\ activity\ (\%) = \frac{Ablank - Asample}{Ablank} \times 100$$

Where A is the absorbance at 515 nm.

Data Analysis

The data were analyzed using one-way ANOVA in IBM SPSS (version 22, IBM Corp.). When significant differences were detected, Duncan's multiple range test was applied at a 5% significance level.

RESULTS AND DISCUSSIONS

Physicochemical Characteristics of Indonesian Kefir

The climate variations across different regions of Indonesia can influence the kefir fermentation process. Temperature, humidity, and environmental conditions play a key role in microbial activity during fermentation. In warmer regions, such as tropical areas, fermentation tends to be faster due to higher microbial activity. Fermentation is slower in cooler areas, leading to kefir with distinct flavor and texture profiles. Additionally, humidity affects microbial growth, which influences the microbiota composition and properties of kefir. The type of milk used also affects kefir's nutritional content, fatty acid composition, protein, and microbial community. For example, kefir made from goat milk tends to have a sharper taste and higher fat content than kefir made from cow milk. Fermentation practices in Indonesia also vary, depending on local traditions and techniques. Longer fermentation times can produce kefir with a more acidic taste and higher lactic acid content, whereas shorter fermentation produces a milder, sweeter kefir. These varying fermentation practices affect the levels and types of phenolics, flavonoids, and other bioactive components in kefir, ultimately influencing its potential health benefits.

Variations in the sampling regions of kefir grains resulted in different outcomes regarding the physicochemical characteristics of kefir (Table 2).

Table 2: Physicochemical characteristics (mean ± S.D., n = 5) of kefir from different regions of kefir grains.

Treatment	Moisture (%)	Viscosity (cP)	Ash (%)	Lactose (%)	Protein (%)	Fat (%)
KG1	90.88 ±0.21^{ab}	89.20 ±2.03^{b}	0.57 ±0.02^{e}	3.48 ±0.20^{a}	3.35 ±0.06^{a}	4.30 ±0.05^{d}
KG2	91.13 ±0.04^{bc}	85.33 ±1.53^{b}	0.55 ±0.01^{e}	3.90 ±0.05^{b}	3.39 ±0.09^{a}	3.45 ±0.03^{c}
KG3	91.43 ± 0.34^{c}	73.63 ±2.12^{a}	0.44 ±0.01^{d}	4.20 ±0.38^{ab}	3.50 ±0.11^{a}	3.44 ±0.02^{c}
KG4	91.19 ±0.12^{bc}	91.73 ±2.76^{bc}	0.14 ±0.00^{a}	4.30 ±0.07^{c}	3.52 ±0.10^{a}	3.47 ±0.01^{c}
KG5	90.66 ±0.07^{a}	128.67 ±1.53^{d}	0.41 ±0.03^{c}	4.44 ±0.07^{d}	3.35 ±0.10^{a}	3.34 ±0.01^{b}
KG6	90.59 ±0.29^{a}	100.47 ±2.96^{c}	0.23 ±0.01^{b}	4.51 ±0.32^{d}	3.35 ±0.02^{a}	2.28 ±0.01^{a}

Note: a,b,c,d,e: Mean with different superscript letters within a column showed a significant difference ($P < 0.05$).

Table 2 explained that the water content of kefir differs significantly based on the source of kefir grains. The lowest water content was observed in kefir grains from North Sumatra (KG5) and West Sumatra (KG6) with values of 90.66±0.07% and 90.59±0.29%, respectively. The fermentation process affected the water content of kefir that resulted acid and changes the structure and composition of the milk. During fermentation, kefir grains also form a gel or milk coagulum. Lactic acid bacteria cause casein pro-

tein to coagulate, forming a denser structure that can hold water. Rosa *et al*. (2017) stated that separating the liquid phase (whey) from the kefir mass increased as fermentation progressed, reducing the water content. The gel structure formed during kefir fermentation helps to hold most of the water in the kefir matrix that affects its texture and water content. As a result, the differences in water content observed in this study are closely related to the viscosity of kefir. The microbial composition and activity in kefir grains directly influence the water content of the final product by regulating the water balance. Ferawati *et al*. (2024) reported that certain microorganisms produce exopolysaccharides that increase the viscosity of kefir and increase water retention in the fermentation matrix. The water content of kefir in this study was higher than that of Otles and Cagindi, (2003) who reported a value of 87.5%.

The ash content of kefir fermented with different kefir grains showed significant differences. The lowest ash content was found in kefir fermented with kefir grains from Central Java (KG4), at 0.14±0.00%, while the highest ash content was found in kefir fermented with kefir grains from East Java (KG1), at 0.57±0.02%. Differences in kefir grains caused variations in the ash content of the kefir. Kefir grains contained a variety of microorganisms that can contribute to releasing minerals from milk components during fermentation. Certain microorganisms, such as lactic acid bacteria, can affect the bioavailability of minerals, changing the mineral composition in kefir and ultimately affecting the ash content. Zhang *et al*. (2024), reported that certain organic acids produced during fermentation may bind certain minerals that affects the accumulation of minerals in the kefir matrix and, therefore, causes differences in ash content. The ash content of kefir observed in this study was lower than the values reported by Wszolek *et al*. (2001), who documented ash content of 0.7-1.1%.

The lactose content of kefir in this study was found to be the lowest in East Java (KG1), that was 3.48 ± 0.20%, and the highest in North Sumatra (KG5) and West Sumatra (KG6) at 4.44 ± 0.07% and 4.51 ± 0.32%, respectively. The lactose content in kefir showed significant variation among various kefir grains. Lactic acid bacteria and yeast in kefir grains play an essential role in the effective breakdown of lactose during fermentation. Certain strains of lactic acid bacteria produce the lactase enzyme, which primarily hydrolyzes lactose into glucose and galactose. The greater the activity of this enzyme, the lower the residual lactose content in kefir after fermentation (McGovern *et al*., 2024). Furthermore, the quality and health of kefir grains significantly affect their ability to ferment lactose. Healthier and more active kefir grains are more efficient in lactose fermentation, while less viable kefir grains may exhibit reduced fermentation efficiency, resulting in higher residual lactose levels in kefir (Al-Mohammadi *et al*., 2021). The lactose content of kefir in this study is comparable to the results reported by Arslan (2015), who documented a range between 4.0% and 6.0%.

In this study, the protein content of kefir did not vary significantly despite being fermented with kefir grains sourced from different regions. However, during fermentation, kefir grains produced proteolytic enzymes that break down milk proteins into peptides and amino acids. Additionally, the microorganisms within the kefir grains convert lactose into lactic acid during fermentation. This process can alter the protein structure in milk, thereby enhancing protein digestibility. Kefir grains also interact with other components in milk, such as fats and carbohydrates, which can influence the stability and bioavailability of protein in the final product (de Sainz *et al*., 2019). The protein content of kefir in this study was similar to the value reported by Sarkar (2007), namely 3.0%.

Significant differences in kefir fat content were observed in this study because of the use of different kefir grains. Kefir fermented with West Sumatra kefir grains (KG6) showed the lowest fat content, measured at 2.28 ± 0.01%. In contrast, the highest fat content was found in the East Java sample (KG1), recorded at 4.30 ± 0.05%. Kefir grains consist of various microorganisms capable of hydrolyzing lipids in milk. This fermentation process affects the fat composition, which affects the amount and type of lipids produced. Some bacterial strains in kefir grains produce lipase enzymes that hydrolyze triglycerides into free fatty acids, thereby affecting the fat content of kefir. Fermentation of milk with different kefir grains produces kefir with varying fat content due to the diverse microbial activities involved in the breakdown of lipid components. Overall, kefir grains determine the fat content of kefir through complex interactions between microorganisms, raw materials, and fermentation conditions (Lu *et al*., 2018). The kefir fat content observed in this study was higher than that reported of kefir sourced from Brazil, as shown by Magalhaes *et al*. (2011) that was recorded at 2.34%.

The total titratable acidity (TTA) and pH values of kefir in this study varied depending on the kefir grains used (Figure 2). The lowest pH value was found in kefir produced using kefir grains from West Java (KG3) at 3.50±0.10, while the highest pH was found in kefir from West Sumatra (KG6) at 4.13±0.12. In contrast, the lowest TTA value was recorded in kefir fermented with North Kalimantan grains (KG2) at 1.39±0.03%, while the highest TTA was found in kefir from East Java (KG1) at 1.61±0.01%. Kefir grains sourced from various regions showed microbial diversity and variations in species composition. Kefir grains contain various microorganisms, including Lactobacillus, Lactococcus, Acetobacter, and yeast. During fermentation, these microorganisms produce lactic acid, acetic acid, and other com-

pounds that reduce the pH of the milk. The availability and specific composition of microbes in kefir grains influence the rate and extent of acid production that determines the final pH of kefir (Biçer *et al.*, 2024).

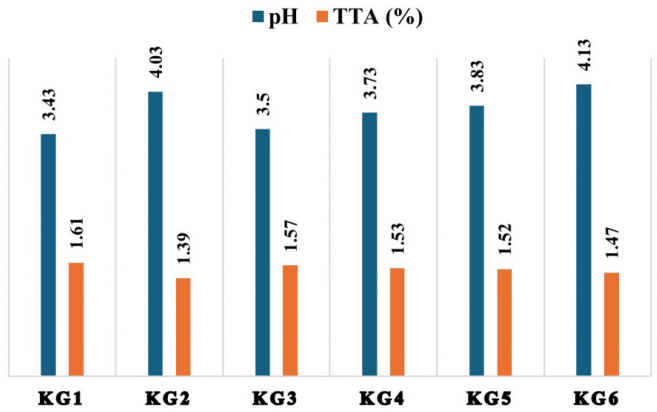

Figure 2: pH values and TTA of kefir from different regions of kefir grains.

The nutrients present in milk, such as lactose, influence microbial activity in kefir grains. Lactose is converted into lactic acid by lactic acid bacteria that contributes to a decrease in pH. Kefir grains with a higher capacity to ferment lactose produces kefir with a lower pH. Environmental factors such as temperature and oxygen levels can also affect microbial activity during fermentation. Higher temperatures usually accelerate fermentation, resulting in a faster decrease in pH (Bourrie *et al.*, 2023). The synergy between lactic acid bacteria and yeast affects the fermentation and acid production rate that causes variations in kefir pH depending on the type of kefir grains used. Furthermore, some microorganisms in kefir grains also produce other organic acids, such as acetic acid, contributing to increase TTA. Variations in microbial composition within kefir grains may influence the quantity of acid produced during fermentation. Strains of microorganisms that are more active in producing lactic or acetic acid may lead to higher TTA values. Lactic acid is the primary organic acid produced during kefir fermentation, typically increasing from 0.1% to 1.0% (v/v) during the process, alongside a 20%-30% (w/v) reduction in lactose (Kesenkas *et al.*, 2017). According to Walsh *et al.* (2016), small amounts of formic acid, succinic acid, hippuric acid, pyruvate, acetate, propionate, and butyrate are also found in kefir. Therefore, variations in microbial composition, fermentation conditions, and microbial interactions will ultimately affect the TTA value of kefir.

Microbiological Properties

The results of this study (Table 3) showed significant differences in the total colonies of lactic acid bacteria (LAB) and yeast in kefir fermented using kefir grains sourced from various regions. The lowest total LAB colonies were found in kefir from East Java (KG1), at 8.36 Log CFU/mL, while the highest were found in kefir from Central Java (KG4) and North Sumatra (KG5), at 10.32 Log CFU/mL and 10.29 Log CFU/mL, respectively. Kefir grain samples cultivated in East Java (KG1) were originally brought from Russia to Indonesia in 1986 and have been used for kefir production ever since. Various fermentation methods and types of milk were used for this kefir fermentation. Climate change and environmental influences are clearly seen to affect the diversity of kefir grain microbiota. According to Van Wyk (2019), the dominant lactic acid bacteria species found in kefir grains from Russia are *Lactobacillus helveticus*, *Lactobacillus kefir*, and *Lactobacillus parakefir*. The types and proportions of microorganisms present in these kefir grains vary depending on their origin and the environmental conditions in which they thrive, such as temperature, humidity, and available nutrients. Several studies showed that kefir grains from different regions had varying numbers and types of microbes. For instance, grains from tropical climates tend to exhibit higher populations of lactic acid bacteria due to the environmental temperatures that favor microbial growth. Arslan (2015) reported that kefir grains from Central Asian mountain regions produced higher total LAB counts than those from cooler climates. The total LAB counts observed in this study were higher than those reported by Bengoa *et al.* (2018), who recorded 9 Log CFU/mL.

Table 3: Microbiological properties (mean ± S.D., n = 5) of kefir from different regions of kefir grains.

Treatment	Lactic Acid Bacteria (Log CFU/mL)	Yeast (Log CFU/mL)
KG1	8.36 ± 0.07^{a}	6.78 ± 0.16^{b}
KG2	9.39 ± 0.03^{b}	6.94 ± 0.08^{b}
KG3	8.57 ± 0.16^{ab}	7.86 ± 0.10^{c}
KG4	10.32 ± 0.07^{c}	6.98 ± 0.06^{b}
KG5	10.29 ± 0.07^{c}	6.02 ± 0.42^{a}
KG6	9.17 ± 0.08^{b}	7.88 ± 0.14^{c}

Note: a,b,c,d,e: Mean with different superscript letters within a column indicate a significant difference ($P < 0.05$).

The yeast colony count varied significantly in kefir fermented with kefir grains from different regions. The lowest total yeast was found in kefir from North Sumatra (KG5) that was 6.02 Log CFU/mL, and the highest in West Java (KG3) and West Sumatra (KG6), that were 7.86 Log CFU/mL and 7.88 Log CFU/mL respectively. Meanwhile, the composition and quantity of yeast in kefir grains varied depending on their origin. The local environment could cause this variation, certain kefir grain species, or the fermentation process. Kefir grains cultivated in North Sumatra (KG5) were initially brought to Indonesia from

China in 2010 and have been continuously used in kefir fermentation. The diversity of milk types and their nutritional and temperature differences affect the diversity and viability of yeast in kefir grains. According to Van Wyk (2019), the dominant yeast species found in Chinese kefir grains are *Kazachtania unispora*, *Kluyveromyces marxianus*, *Saccharomyces cervisiae* and *Kazachtania exigua*.

Kefir grain had the highest total yeast from West Sumatra (KG6) that was initially brought to Indonesia from Brazil in 2007 and it is still cultivated. Kefir grain from West Sumatra has been used to ferment goat milk and cow milk. In addition, this kefir grain's fermentation time varies between 24 hours to 48 hours. The difference in climate and fermentation techniques is clearly seen to affect the diversity and number of yeast colonies in kefir grains. According to Magalhaes *et al.* (2011), the dominant yeast species in Brazilian kefir grains are *Kazachtania aerobia*, *Saccharomyces cervisiae* and *Lachancea meyersii*. The specific content of each yeast strain in kefir grains affects the total yeast present in the final kefir product. Kefir grains with higher yeast concentrations, such as *Saccharomyces cerevisiae*, tend to produce kefir with higher yeast content (Van Wyk, 2019). The number of yeast colonies observed in this study was similar to that reported by Chen *et al.* (2015), who recorded 7 Log CFU/mL.

Functional Properties of Kefir in Human Health

Kefir, a fermented dairy product, has long been recognized for its potential health benefits, primarily attributed to its bioactive compounds, especially phenolic compounds. These bioactive substances contribute significantly to the functional properties of kefir, especially in terms of antioxidant activity and its potential to inhibit enzymes such as alpha-glucosidase. The functional properties of kefir have gained considerable attention due to their implications for human health, especially in preventing and managing chronic diseases, including metabolic disorders and oxidative stress-related conditions.

Phenolic compounds, a group of derived bioactive molecules, are present in various foods and beverages, including kefir. These compounds are recognized for their antioxidant properties, which protect the body from oxidative damage caused by free radicals. In kefir, phenolic compounds are likely produced during fermentation through microbial activity, which enhances their concentration. Research indicates that kefir contains diverse phenolic acids, flavonoids, and polyphenols, contributing to its antioxidant capacity.

Table 4 showed significant differences in the total phenolic content (TPC) of kefir produced from kefir grains sourced from various regions. The lowest TPC was observed in kefir from North Kalimantan (KG2), with a value of 125.23±3.31 mg GAE/g while the highest TPC was recorded in kefir from North Sumatra (KG5), at 309.47±3.44 mg GAE/g. Kefir grains play a crucial role in determining the total phenolic content of kefir. The fermentation process contributed to producing organic acids and alcohols and increases the levels of bioactive compounds such as phenolics. Microorganisms in kefir grains can produce certain enzymes such as β-glucosidase, which release phenolic compounds from their precursors. During fermentation, these enzymes hydrolyze phenolic glycosides in fermented milk, converting them into aglycones, phenols' active and measurable form. Studies showed that kefir fermented with kefir grains exhibits significantly higher phenolic content than without grains (Du *et al.*, 2023). In addition to releasing phenols from milk components, microorganisms in kefir grains may also convert non-phenolic compounds into bioactive phenolic forms. Furthermore, Constantin *et al.* (2023) reported that *Lactobacillus plantarum* strains in kefir grains were more effective in producing phenolic compounds than *Lactobacillus acidophilus*. The microbial composition of kefir grains plays a vital role in determining the final phenolic content of the product. According to Perna *et al.* (2018), fermentation using kefir grains increased the total phenolic content of kefir by up to 30%, with bioconversion of compounds such as cinnamic acid and chlorogenic acid in milk contributing to this increase.

Table 4: Total phenolic content, antioxidant capacity, and α-glucosidase inhibitory activity (mean ± S.D., n = 5) of kefir from different regions of kefir grains.

Treatment	TPC (mg GAE/g)	% Scavenging of DPPH	% Glucosidase inhibition
KG1	195.38±1.74^{b}	13.58±2.47^{a}	17.89±1.10^{a}
KG2	125.23±3.31^{a}	13.01±1.78^{a}	19.65±0.73^{ab}
KG3	197.50±1.31^{b}	15.43±3.18^{a}	16.27±1.54^{a}
KG4	263.56±3.65^{c}	19.08±2.85^{a}	22.26±3.18^{bc}
KG5	309.47±3.44^{e}	26.47±3.15^{b}	30.52±3.52^{d}
KG6	294.92±4.98^{d}	19.66±1.34^{a}	24.48±1.46^{c}

Note: a,b,c,d,e: Mean with different superscript letters within a column indicate a significant difference (P < 0.05).

The antioxidant activity of kefir plays a crucial role in its ability to promote human health. Antioxidants neutralize free radicals—unstable molecules that can damage cells and tissues, contributing to the aging process and the development of various diseases. The presence of phenolic compounds in kefir has been shown to increase its antioxidant capacity, helping to reduce oxidative stress. This antioxidant activity is particularly significant in the context of diseases such as cardiovascular conditions, neurodegenerative diseases, and diabetes, where oxidative stress is a key factor in the progression of the disease. Kefir grains from

various regions exhibited varying antioxidant activity, with the highest activity observed in kefir grains from North Sumatra (KG5) at 26.47±3.15%. Kefir grains are essential in inhibiting free radicals by producing bioactive compounds during fermentation. These compounds contribute to antioxidant activity by neutralizing free radicals and preventing oxidative damage to body cells. The antioxidant activity of kefir microorganisms is associated with the enzyme *β*-glucosidase that breaks down phenolic glycosides in milk into active aglycone forms with high antioxidant potential. According to Erdogan *et al.* (2018), various bioactive compounds such as bioactive peptides, organic acids, exopolysaccharides, and phenolic compounds produced during kefir fermentation can clean and neutralize free radicals. A study by Yilmaz-Ersan *et al.* (2018) reported an increase in antioxidant activity by 40% after 48 hours of kefir fermentation.

Another significant functional property of kefir is its ability to inhibit the activity of alpha-glucosidase, an enzyme involved in the digestion of carbohydrates. Alpha-glucosidase breaks down complex carbohydrates into simple sugars like glucose, which are then absorbed into the bloodstream. In individuals with diabetes, the rapid breakdown of carbohydrates can lead to spikes in blood glucose levels. Therefore, inhibiting alpha-glucosidase activity is an effective strategy for managing blood sugar levels, especially after meals. Research has demonstrated that kefir can inhibit alpha-glucosidase activity, thus slowing down the digestion and absorption of carbohydrates. This results in a more gradual increase in blood glucose levels, which benefits individuals with type 2 diabetes or those at risk of developing the disease. The ability of kefir to modulate blood sugar levels through enzyme inhibition makes it a promising dietary intervention for improving glycemic control and reducing the risk of diabetes-related complications.

Kefir produced using kefir grains from various sources exhibited varying levels of α-glucosidase inhibitory activity. The highest activity was observed in kefir grains from North Sumatra (KG5), with a value of 30.52±3.52% while the lowest was from West Java (KG3), at 16.27±1.54%. During fermentation, microorganisms in kefir produce various bioactive compounds, such as bioactive peptides, exopolysaccharides, organic acids, and polyphenols. These compounds have been shown to exhibit α-glucosidase inhibitory activity. A study by Yusuf *et al.* (2021) showed that polyphenol compounds inhibit the enzyme by binding to it and changing its active conformation, thereby reducing its effectiveness in breaking down carbohydrates into glucose. Peptides produced from the degradation of milk protein by microorganisms in kefir grains also have the potential to inhibit *α*-glucosidase. According to research by Luo *et al.* (2023), bioactive peptides produced during kefir fermentation can interact with the enzyme's active site, thereby preventing carbohydrate substrates from accessing the enzyme. Yusuf *et al.* (2021) stated that Lactobacillus spp. isolated from Indonesian kefir grains showed the capacity to produce compounds with varying levels of α-glucosidase inhibitory and antioxidant activities. In particular, the highest antioxidant activity was observed in *Lactobacillus kefiri* JK5 and *Lactobacillus kefiri* JK17, with percentages of 44.31% and 41.57%, respectively.

CONCLUSIONS AND RECOMMENDATIONS

Kefir produced in Indonesia is primarily fermented using kefir grains that were originally sourced from various countries. Although the kefir grains examined in this study have been cultivated in Indonesia for at least five years, they exhibit notable variations in nutritional composition, microbial content, total phenolic content, antioxidant activity, and α-glucosidase inhibition. Despite these differences, all kefir samples complied with the quality standards for fermented foods, with kefir derived from North Sumatra (KG5) demonstrating the most favorable characteristics among the six regions studied.

These findings have significant implications for both kefir producers and consumers in Indonesia. The observed variations in kefir properties suggest that regional factors, including environmental conditions and fermentation practices, may influence its functional potential. A deeper understanding of these differences can aid in optimizing production techniques to enhance kefir's nutritional and probiotic qualities, thereby improving its commercial viability.

Future research should prioritize in-vivo studies to further substantiate the health benefits of kefir as a functional food. Specifically, subsequent studies should explore its effects on metabolic disorders, gut microbiota modulation, and immune system regulation. Identifying the optimal dosage and assessing the long-term effects of kefir consumption will be crucial for developing evidence-based recommendations for its role in health promotion and disease prevention. Expanding this research will not only provide scientific validation of kefir's functional properties but also support its advancement as a valuable dairy product with health-promoting potential.

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NOVELTY STATEMENT

This study is the first to compare and analyze the nutritional and microbial profiles of kefir from diverse regions in Indonesia.

AUTHOR'S CONTRIBUTIONS

Ferawati: designed the research concept, conducted the experiments, and analyzed the data.
Yetti Marlida and Sri Melia: composed and wrote the script.
Arief: conducted the laboratory work and visualized the data.

Conflict of Interest

All authors declare that there is no conflict of interest.

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