

Physicochemical and microbial evaluation of Kefir produced with sesame seed (*Sesamum indicum* L.) extract as a goat milk substitute

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ABSTRACT

Kefir is one of traditional beverage made by milk fermentation using kefir grain. Kefir can be produced with the addition or substitution with plant based ingredients to enhance it nutritional and functional properties. the objective of this research to investigate the influence of substituting with various concentration of sesame seed extract on physicochemical and microbiological characteristic of kefir goat milk. The present study was consisting of five concentrations of sesame seed extract (10, 20, 30, 40 and 50%) using completely randomized design. The parameter assessed were pH, syneresis, WHC, total Lactic Acid Bacterial (LAB) count, total yeast count and antibacterial activity (*Escherichia coli* and *Staphylococcus aureus*). The experimental data statically analyzed using analysis of variance and followed with Duncan test. The result indicated that increasing sesame seed extract causes decrease in pH and water holding capacity while syneresis increased. The finding of the research revealed that with increasing the sesame seed concentration resulted in reduction of pH and WHC while increased syneresis of kefir. Total LAB increase until 40% substitution of sesame seed extract (15.73×10^6 CFU/g) and total yeast count fluctuated among treatment with the highest yeast in 20% substitution (25.43×10^6 CFU/g). Kefir with sesame seed extract demonstrated antibacterial activity against *E. coli* and *S. aureus* with the highest activity from substitution 30% (*E. coli*: 2.15cm) and 40% (*S. aureus*: 2.18cm). Thus, sesame seed extract demonstrated for substituting goat milk for making kefir with optimal concentration level extract 30-40%.

Introduction

Kefir is widely known fermented milk product around the world (Leite *et al.*, 2013). Several beneficial compounds presented in from microorganism presented in kefir as vitamins, minerals, essential amino acids that aid in homeostasis, hydrolyze lactose and degrade proteins, resulting in nutritious and easily digestible food (Ahmad *et al.*, 2013). The advantage associated with kefir are antioxidant, antibacterial activity and galactosidase activity, anti-hypercholesterolemic, anti-carcinogenic and anti-tumor effects (Farag *et al.*, 2020). Health promoting benefit of kefir are inhibiting the growth of pathogenic bacteria which can cause gastrointestinal diseases by producing antibacterial compounds such as bacteriocin, antibiotics or hydrogen peroxide. The presence of lactic acid bacteria in kefir have the ability to prevent urinary infection, increase immunity and reduce heart disease (Azizi *et al.*, 2022)

Kefir is made using milk and starter kefir grain which consist of LAB and yeast which proliferate synergically during the fermentation (Güzel-Sydim *et al.*, 2011). The microorganism found in kefir grain consist of various of LAB (*Lactobacillus kefir*, *Lactobacillus parakefir*, *Lactobacillus paracasei*, *Lactobacillus kefiranofaciens* ssp. *kefiranofaciens*, *Lactobacillus kefiranofaciens* ssp. *kefirgranum*, *Lactobacillus plantarum*), *Leuconostoc*, *Lactococcus*, *Acetobacter*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, *Saccharomyces unisporus*, and *Saccharomyces exiguus* (CODEX 2003).

Kefir is produced using different types of milk sources including cow, goat, buffalo, sheep, camel and donkey (La Torre *et al.*, 2024). Milk commonly used to make kefir is goat milk because the optimum nutritional content such as protein, amino acid, short-chain fats and easily digestible biopeptides (Park *et al.*, 2007). Compare with cow's milk, goat milk contain fat with smaller globules, a slight laxative impact and highly digestible protein. Goat milk also produces lactose with lower content, distinct flavor profile including soft texture and aroma (Karni, 2023).

For further the functional properties of kefir, the use of plant based as food substitute has been proposed. Several plant-based ingredients have been studied for kefir production. such as soy, almond seed and rice extracts. One of the plant based ingredients which is used in this

research and can be utilized is sesame seed. A significant food for diets, sesame seed are known for their health advantage including supporting metabolic balance, cardiovascular health and cancer prevention. the bioactive constituent found in sesame seed are lignans, tocopherols and polyunsaturated fatty acids which is improving the nutritional composition and health promoting properties (Mn *et al.*, 2012; Sharma *et al.*, 2021; Mostashari and Khaneghah, 2024).

Several previous studies have shown that sesame seed extract can be used in the formulation of beverages or functional food products. Sesame oil has been reported by El-Sayed (2015) as a new functional beverage rich in total phenolic compounds and high in antioxidant activity which increased with increase of sesame oil addition. Sesame seeds have been used in fermented dairy products, one example being yogurt. Sesame seeds have been used in fermented dairy products, one example being yogurt

Sesame seed have been used to produce yogurt with enhanced functional and the nutritional composition particularly the presence of omega-3 and omega-6 content. Yogurt produced with the addition of sesame seed has a desired microbiological and physical characteristic and organoleptically acceptable (Afaneh, 2011; Finco *et al.*, 2011). The addition of plant based material for fermentation product affect the physical characteristics, water holding capacity and syneresis (Tosif *et al.*, 2024). Thus, the objective of this research was to investigate the influence of substituting sesame seed extract on physicochemical and microbiological characteristic of kefir goat milk.

Materials and methods

Sesame seed water extract preparation

Sesame seed was extracted using water according to Suhartatik and Widiarti (2016) with modification: First sesame seed were cleaned and subsequently steamed for 10 minutes. Then, they were blended with water in 1:2 volume per volume ratio. The resulting mixture was filtered and pasteurized at 110°C for 10 minutes.

Kefir Preparation

The process involved mixing sesame seed extract with goat milk according to their respective concentration 10, 20, 30, 40 and 50%. The mixture was thermally treated at 85°C for 15 minutes and cooled at 40°C and then inoculated with kefir grains at 5% of the total mixture, then incubated at 30°C for 24 hours (Rossi *et al.*, 2016).

The preparation of sesame seed extract is based on the method by Suhartatik and Widiyanti (2016)

pH value

The pH value was measured using a pH meter. Before use, the device was first calibrated with buffer solutions representing low pH (4.00) and neutral pH (7.00). After that, the device was turned on and the pH meter electrode was immersed in the sample, and the pH value was displayed on the pH meter.

Syneresis

Syneresis was measured using the drainage method based on the procedure by Berlianti *et al.* (2022) with slight modifications as follows: A sample of 10 ml was poured into a jar equipped with a funnel and filter paper, left for 30 minutes. The separated liquid was measured, and syneresis was calculated using the formula:

$$\text{Syneresis(\%)} = \frac{B}{A} \times 100$$

Note:

A = Initial Volume

B = Whey Volume (the liquid that separates)

Water holding capacity (WHC)

WHC was measured based on the method of Berlianti *et al.*, (2022) with slight modifications as follows: A 2 ml sample was weighed, then placed into an Eppendorf tube. Centrifuged at a speed of 3000 rpm for 10 minutes at room temperature. The separated whey was weighed and calculated using the formula:

$$\text{WHC(\%)} = \frac{(A-B)}{A} \times 100$$

Note:

A = Initial Volume

B = Whey Volume (the liquid that separates)

Total lactic acid bacteria and total yeast

One mL kefir sample from each treatment was transferred to 9 mL of sterile saline solution and serial dilutions were prepared by mixing 1 mL of the homogenized sample with 9 mL of sterile saline solution. Total LAB were enumerated by plating samples on Lactobacillus MRS Agar after aerobic incubation at 37°C for 24 h. Total yeast were counted by plating serial dilution on Malt Extract Agar after incubation at 37°C for 48 h. The formed colonies were counted and expressed as colony forming units of the suspension (CFU/mL).

Antibacterial activity

Antibacterial activity was detected by the spot on lawn method with some modifications (Cadirci and Citak, 2005). All test bacteria were cultured on Mueller-Hinton broth and incubated at 37°C for 24 h. The culture broth was diluted using MHB to 0.5 McF and spread onto Mueller-Hinton agar using sterilized cotton swabs. Twenty microliters of each kefir supernatant were directly dropped onto the surface of the MHA. The plates were incubated for 24 h at 37°C, and the inhibition zone was observed. The presence of a clear zone at the site of kefir inoculation was considered as total inhibition. As a control chloramphenicol was diluted

with sterilized distilled water.

Statistical analysis

The treatments established are: P1= 10% Sesame Seed Extract; P2= 20% Sesame Seed Extract; P3 = 30% Sesame Seed Extract; P4= 40% Sesame Seed Extract; P5= 50% Sesame Seed Extract. The observation data were analyzed using analysis of variance to determine the effect between treatments. Then, further testing was conducted using Duncan's multiple range test with $p < 0.05$ to determine the differences between treatments.

Results

Kefir pH values

Based on the analysis of variance, the substitution of goats' milk with sesame extract has a significant effect ($p < 0.05$) on the pH value. The pH values range from 4.45 to 4.98. The substitution of sesame extract at 10-30% is significantly different from the treatments with 40% and 50% sesame extract.

Syneresis and WHC of Kefir

The physical characteristics that affect the quality of kefir include syneresis and Water Holding Capacity, presented in Table 1. From the data in Table 1, syneresis data was obtained with a range of 34.2 – 44.4%, while WHC ranged from 56.5 – 66.7%. The analysis of variance of kefir shows that syneresis and WHC are significantly influenced by the substitution of sesame extract ($p < 0.05$) and differ significantly among treatments. Syneresis increased with the increasing substitution of sesame extract, whereas WHC, on the contrary, decreased with the increasing substitution of sesame seed extract.

Table 1. Syneresis and Water Holding Capacity of Kefir.

Treatment	Syneresis (%)	WHC (%)
10%	34.2 ^a ±0.7	66.7 ^a ±1.2
20%	39.4 ^b ±0.5	61.5 ^b ±0.9
30%	40.5 ^b ±0.3	60.6 ^b ±0.8
40%	40.0 ^b ±0.7	60.3 ^b ±1.0
50%	44.4 ^c ±0.6	56.5 ^c ±0.8

^{a-c} Different superscripts in the same row represent significant differences ($p < 0.05$).

Lactic acid bacteria and yeast

The number of lactic acid bacteria and kefir yeast with sesame extract substitution is presented in Table 2. The number of lactic acid bacteria ranged from 4.25×10^6 – 12.62×10^6 CFU/mL, while the total yeast count ranged from 17.25×10^6 – 25.43×10^6 CFU/mL. There was an increase in the number of LAB along with the increase in sesame extract concentration from 10% to a peak at 40% (15.73×10^6 CFU/mL), before then decreasing at a concentration of 50% (12.62×10^6 CFU/mL). Based on the analysis of variance, it was found that sesame extract substitution had a significant effect ($p < 0.05$) on the total LAB and total yeast.

Kefir antibacterial activity

The antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* from kefir supplemented with sesame extract is presented in Table 3. The inhibition zone diameter for *E. coli* ranged from 1.12 to 2.15 cm, while for *S. aureus* it ranged from 1.12 to 2.18 cm. The results of variance analysis test indicated that the substitution of sesame extract in kefir has a significant effect ($p < 0.05$).

Table 2. Total BAL and Yeast Kefir.

Treatment	Total BAL (x10 ⁶ CFU/mL)	Total Yeast (x10 ⁶ CFU/mL)
10%	4.25 ^a ±0.55	17.25 ^b ±0.75
20%	8.13 ^c ±0.64	25.43 ^a ±0.32
30%	11.35 ^b ±1.24	15.76 ^b ±0.66
40%	15.73 ^a ±0.44	17.54 ^b ±0.23
50%	12.62 ^b ±1.22	22.15 ^a ±0.75

^{a-c} Different superscripts in the same row represent significant differences (p<0.05).

Table 3. Diameter of the inhibition zone of kefir.

Treatment	Diameter of Inhibition Zone <i>E. coli</i> (cm)	Diameter of Inhibition Zone <i>S. aureus</i> (cm)
10%	1.94 ^b ±0.54	1.12 ^b ±0.64
20%	1.47 ^b ±0.47	1.15 ^b ±0.53
30%	2.15 ^a ±0.60	1.21 ^b ±0.83
40%	1.12 ^c ±0.43	2.18 ^a ±0.76
50%	1.15 ^c ±0.15	1.82 ^a ±0.89

^{a-c} Different superscripts in the same row represent significant differences (p<0.05).

Discussion

pH value of Kefir is acidic and has a pH ranging from 3.87 to 4.45, depending on the kefir grains used and the fermentation conditions (Agustina *et al.* 2013). The pH of goat milk kefir substitute with sesame seed extract was decreased with increasing the substitution concentration. Kefir with substitution 10%, 20% and 30% exhibited significantly higher pH values (4.98, 4.76 and 4.49, respectively) than the substitution with 40% and 50% (both 4.45). The substitution of sesame extract lowers the pH of kefir because sesame extract contains nutrients such as carbohydrates, proteins, and bioactive compounds that can be utilized by lactic acid bacteria during the fermentation process. The nutrients in sesame extract stimulate the growth and activity of lactic acid bacteria in kefir grains, thereby increasing the organic acids produced during fermentation, especially lactic acid.

The fermentation process, driven by LAB convert lactose into acid. it has been observed that increasing sesame seed extract enhancing the fermentation and decreasing the pH (Barboza *et al.*, 2016; Margareth *et al.*, 2020).

Syneresis is defined as the percentage of water released as a result of decrease of protein network to retained moisture during draining. The higher water release or high syneresis indicate a weak protein network, which reduces water holding capacity or ability to hold water (Agustina *et al.* 2013). Adding sesame seed extract appears to increase syneresis and weakening the gel, which may be caused by decreasing milk protein, specifically casein in the 30, 40 and 50% substitution treatment. conversely, at 10% and 20% substitution, syneresis remain low, demonstrating that the protein matrix is stable to resist releasing liquid from kefir. Stable protein structure is key in reducing syneresis, as it able to bind more water and create a stable gel network. The level syneresis in kefir is influenced by the fermentation process and the composition of kefir grain including LAB and yeast. Different fermentation altered different of syneresis level as reported by Nielsen *et al.* (2014).

Adding more sesame seed extract reduced WHC of kefir, possibly because the protein and polysaccharides from sesame seed extract were not effective at retaining water. Increasing WHC result in thick kefir and low syneresis (whey separation). water holding capacity was determined by protein and polysaccharides network to retain water during storage.

The findings of Ghadge *et al.* (2008) revealed a correlation between thicker yogurt and reduce syneresis. The use of functional ingredients such as protein, fibers or polysaccharides can also improve water holding

capacity of the product. This is consistent with Wals *et al.* (2010) in which product with high WHC exhibit low syneresis. This is because of a denser protein network that improve water binding.

Kefir made with 40% substitution of sesame seed extract showed an increase in total LAB, indicated that LAB can utilize carbohydrates, amino acid and minerals from the extract for their growth during fermentation. Sesame seed extract contains 9.58 g/100g carbohydrates, consisting of D-glucose, D-galactose, D-fructose, raffinose, stachyose, planteose and sesamose (Hedge, 2012). The addition of sesame seed extract increased the fermentation process due to the additional source of carbohydrate which is supplement the lactose in goat's milk. A decline in total LAB was observed at 50% substitution of sesame seed extract, this is presumably due to either high osmotic pressure from sesame seed extract or antimicrobial compounds found in sesame seed that can suppress microbial growth (Dahiya and Nigam, 2023).

The total yeast in the 20% sesame seed extract was high suggesting that the availability of sugar and optimum condition to support yeast growth (Beigbeder *et al.*, 2021). a decrease in total yeast at 30% and 40% substitution of sesame seed extract is suspected because the competition for nutrients with LAB. This is supported with the finding of Cheng *et al.* (2024) who concluded that microbial competition can occur when kefir medium has limited nitrogen content.

The combine and synergy growth of LAB and yeast is essential for developing the uniq characteristics kefir. LAB is responsible for producing lactic acid which lowers the pH of kefir and add probiotic benefits, whereas yeast contributes to the product's aroma and CO₂ production. these finding align with the research of Prado *et al.* (2015), who found that the composition of substract influence the microbial balance during kefir fermentation.

The substitution of sesame seed extract into kefir demonstrated antimicrobial activity against both Gram negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*). The effectiveness of this antibacterial activity was measured by the diameter of the inhibition zone, a wider zone signifies a greater inhibitory capacity.

Adding up to 30% sesame seed extract to kefir enhance the inhibition of *E. coli*. this effect is attributed to a synergistic interaction between phenolic compounds (including sesamin and sesamol), flavonoid and lignans. however, at high concentration of sesame seed (40% and 50%), the antibacterial activity decreases. This is suspected to be the result of the high concentration of phenolic compounds, which may decrease the viability of the LAB and disrupt the microbial balance of the fermentation process. This disruption leads to decrease in the production of antibacterial compounds.

These findings suggest that *S. aureus* (Gram positive) is more susceptible to phenolic compounds and lignans in sesame seed extract than *E. coli* (Gram negative). While Gram positive bacteria possess a thicker peptidoglycan layer, this structure increases their permeability to the lipophilic compounds found in plant extracts.

Conclusion

Kefir with sesame extract substitution up to 50% shows increased antibacterial activity and higher total BAL and yeast but decreases WHC and increases syneresis. The concentration of sesame extract in the range of 30–40% is the optimal point that provides a balance between antimicrobial activity, fermentation microbial viability, and the physical quality of kefir.

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Conflict of interest

The authors have no conflict of interest to declare.

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