Antibacterial activity, physical and chemical properties of sweet corn milk kefir with variation of starter concentration and fermentation time

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Abstract. Sweet corn is a potential raw material for kefir production due to its high carbohydrate content. The purpose of this study was to determine the effect of starter concentration and fermentation time on the properties of sweet corn milk kefir and to find the optimal starter concentration and fermentation time. The kefir was prepared with starter concentrations of 2%, 4%, 6%, with fermentation times ranging from 8, 16, 32, 40, 48, 56, 64, and 72 hours. Organoleptic tests, pH, acid content, and antibacterial activity were studied. The results obtained were analyzed statistically. The results showed that starter concentration and fermentation time significantly affected organoleptic properties, pH, lactic acid content, and antibacterial activity ($P<0.05$). The longer the fermentation time, the lower the pH value, the higher the acid content, and the higher the antibacterial activity. The fermentation time of 64 hours and 4% starter concentration showed a pH value of 3.46 with the highest acid content of 2.107% and an inhibition zone diameter of 11.02 mm. Starter concentration and fermentation time of kefir significantly influenced organoleptic properties, pH, acid content, and antibacterial activity against *V. cholerae*. The optimal kefir was obtained at 4% starter concentration and 64 hours of fermentation time.

1 Introduction

Kefir is fermented milk from grains containing a unique and complex combination of symbiotic bacteria and yeasts [1]. When kefir grains are inoculated into a culture medium, such as milk, they produce acidified fermented milk with mild carbonation and traces of alcohol. During fermentation, lactic acid, bioactive peptides, exopolysaccharides, antibiotics, and several bacteriocins are produced [2], [3].

Animal milk, especially cow's and goat's milk, is commonly used in the production of kefir [4]. However, the availability of animal milk has sometimes been limited and relatively expensive. Also, some people are allergic to animal milk, which requires using a substitute, especially plant-based milk. Milk from sweet corn can be used to make kefir.

Sweet corn has a high carbohydrate and reducing sugar content, making it a potential raw material for making kefir. The high content of reducing sugars in sweet corn can be a source

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of energy for the bacteria that perform the fermentation. In the fermentation process, lactose is a sugar that can be directly broken down by bacteria into lactic acid. However, the sugar content of sweet corn is glucose, fructose and sucrose with a content of 16-18% [5], [6]. In addition to its delicious taste, sweet corn is rich in nutrients and bioactive compounds, including amino acids, unsaturated fats, dietary fiber, vitamins, minerals, phenolics, and other phytochemicals [7], [8]. Corn has a very high starch content (72-73%), namely amylose and amylopectin starch in the ratio of 25-30%: 70-75%. The protein content of corn is quite high, ranging from 8-11%. Corn also contains unsaturated fatty acids, namely omega-9 (oleic acid) and omega-6 (linoleic acid) [9]. Amino acids and vitamins found in corn include vitamins A, B1, B2, and E [7], [10].

Several factors can influence kefir production, including the type of milk and the fermentation process. The use of various starters and the fermentation time may affect the quality of the kefir, altering its nutritional value and the physical properties or texture of the kefir. For almost all fermented beverages made commercially, starter cultures are a necessary ingredient. To induce desired and predictable changes in the final product, microorganisms known as starter cultures are put directly into fundamental ingredients such as milk. These could include greater nutritional value, improved preservation, altered sensory characteristics, and increased economic value [11]. Another of the key variables is the length of fermentation, which affects the kefir's physical (such as viscosity) and chemical (such as pH and acidity) characteristics. The duration of the fermentation process is correlated with the microbial starter's ability to convert substrate into product, which has a major effect on the kefir's characteristics.

Kefir has attracted the interest of the scientific community due to its purported therapeutic properties, which include improving digestion and lactose tolerance, antimicrobial action, antioxidant, and hypocholesterolemic effect [12], [13].The aim of this research was to study the effect of starter concentration and fermentation time on sweet corn milk kefir, especially on the antibacterial activity against *Vibrio cholerae*, and to know the optimal kefir starter concentration and fermentation time in the production of sweet corn milk kefir.

2 Material and Methods

The materials used in this study were sweet corns obtained from Muntilan Magelang Central of Java, skim milk, kefir grains, *Vibrio cholerae,* and NaCl 0.9%. The schematic of the procedure of this study is represented in Figure 1.

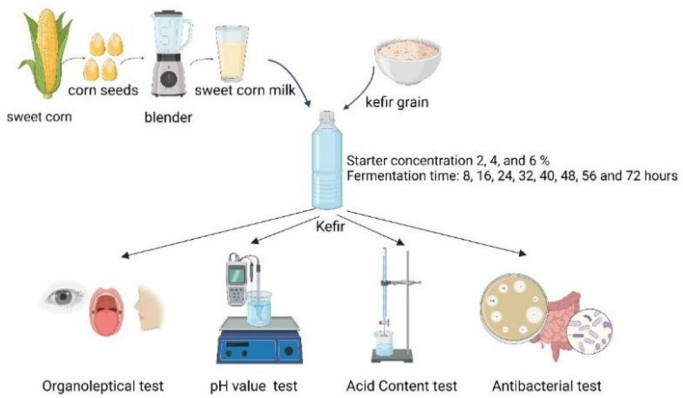

Fig. 1. Schematic of sweet corn milk kefir fermentation production and evaluation. This image was created with BioRender.com under agreement number "CS26TU2DXW".

2.1 Preparation of Sweet Corn Milk

The corn was peeled, then shelled, and weighed approximately 1.5 kg. The corn kernels were boiled with 2 liters of water until they were boiling. After boiling, the corn kernels are drained and cooled. Then blended by adding 4.5 liters of water until smooth, and then filtered to remove the juice. The sweet corn milk filtrate was then pasteurized by heating at 80^{o}C for 30 minutes [14].

2.2 Preparation of Sweet Corn Milk Kefir

Pasteurized corn milk was added with 10% skim milk, then cooled in a glass container until it reached room temperature around 18-25^{o}C. Then 2%, 4%, and 6% kefir seed grains were added and stored for 3 days, and every 8 hours, the organoleptic properties, pH value, acid content, and antibacterial activity were tested [15]. The test was performed for fermentation times of 8, 16, 24, 32, 40, 48, 56, 64, and 72 h. Kefir was filtered to remove the kefir grain, and then the filtrate was kept at 4^{o}C.

2.3 Organoleptical Test

2.3.1 Color test

Approximately 5 ml of kefir was placed in a test tube with a white background, and the color of the kefir was observed. Observations were made descriptively: score 1 if yellow, score 2 yellowish-white, score 3 slightly yellowish-white, score 4 white, and score 5 very white.

2.3.2 Flavor test

Kefir was poured into a glass and tasted for flavor. Score using the descriptive method: score 1 if very not sour, score 2 not sour, score 3 slightly sour, score 4 sour, and score 5 very sour.

2.3.3 Odor test

Kefir was placed in a test tube, approximately 5 ml, and then smelled. Score 1 if the smell is very typical of fermented milk, score 2 if it is typical of fermented milk, score 3 if it is less typical of fermented milk, score 4 if it is not typical of fermented milk; and score 5 if it has the smell of spoiled milk [16].

2.4 Acidity (pH) Test

Sweet corn milk kefir with fermentation times of 8, 16, 24, 32, 40, 48, 56, 64, and 72 hours and concentrations of 2%, 4%, and 6% were tested for acidity level (pH value) using a pH meter. Previously, the electrode was standardized first with buffer 4 and buffer 7, and then the electrode was immersed in the sample (kefir) until the pH was fixed [11].

2.5 Determination of Lactic Acid Content

The determination of the lactic acid content of kefir was carried out by the titrimetric method using a NaOH solution. Sweet corn milk kefir, as much as 5 grams, was put in the Erlenmeyer, and then it was added with 3 drops of the pp indicator. Then it was titrated with 0.1 N NaOH until a stable pink color was formed [17].

$$\text{Lactic acid content (\%)} = \frac{\text{ml NaOH X N NaOHx 90.08}}{\text{sample weight (g)x 1000}} \text{ x 100 \%} \quad (1)$$

2.6 Antibacterial activity test

Previously, a suspension of *V. cholerae* in 0.9% NaCl was prepared to a turbidity comparable to 10^8 CFU/mL and diluted to 10^6 CFU/mL [18]. Sterile cotton swabs were dipped in the bacterial suspension and spread evenly over the surface of Mueller Hinton agar media, then perforated with a 6 mm diameter. Amounts of 50 µL of the sample (corn milk kefir) at concentrations of 2%, 4%, and 6% were added to the wells and then incubated at 37°C for 20–24 hours. The diameters of the inhibition zones were measured with a ruler. The corn milk was used as a negative control and a positive control (disc) containing chloramphenicol 30 µg/mL.

2.7 Data analysis

Quantitative data obtained included the diameter of bacterial inhibition, pH value, and lactic acid content against the length of fermentation time and concentration of kefir seeds, each with three replications. The data obtained were analyzed statistically using the two-way ANOVA-Tukey HSD test. In addition, correlation test analysis was also conducted to determine the relationship between pH, acid content, and inhibition zone using the Pearson test.

3 Results and Discussion

In this study, sweet corn milk was used as the main substrate for kefir production. The advantages of sweet corn milk over cow's milk or soy milk are that the raw material is readily available and the price is not too high. Sweet corn milk contains more fiber, which is good for people trying to lose weight. Sweet corn milk is a novel idea for the production of kefir-based products. The plant-based milk will serve as an alternative to animal-based milk. This study aims to increase domestic consumption of corn, which is currently considered low. Based on this, creating a new corn-based product in the form of healthy kefir is a way to promote health [19].

When making corn milk kefir, 10% skim milk was added because the protein and lactose sugar content of corn milk are still lacking. The lactose content in skim milk stimulates the growth of lactic acid bacteria, which play a role in producing lactic acid or other flavor components, so the kefir tastes sour and has a distinctive aroma of fermented milk [20]. Organoleptic properties greatly affect the level of preference for kefir. Table 1 summarizes the organoleptic properties of sweet corn kefir made with various starter concentrations and fermentation times. The color of kefir was yellow to slightly yellowish white (1.00–3.00). This color was due to the yellow color of vitamin B2 (riboflavin) and carotenoid. During the fermentation process, lactic acid bacteria break down lactose into lactic acid. Lactic acid causes the pH to drop, giving the kefir a sour taste. The higher the concentration and the longer the fermentation, the more acid is produced in the kefir, causing a sour taste [11]. The flavors of sweet corn milk kefir were descriptively slightly sour to very sour (2.67–5.00). Odor is one that determines whether consumers like a food product or not; if the produced smell is good, it will increase the preference for the sweet corn milk kefir. A descriptive evaluation gave an odor rating from very typical of fermented milk to less typical of fermented milk (1.00–2.67). The higher the concentration and the longer the fermentation

time, the sharper the kefir's color, odor, and flavor. However, the flavor and odor of corn milk kefir were in accordance with the kefir standard [21].

Table 1. The score of organoleptic of sweet corn milk kefir

Fermentation Time (hour)	Descriptive Value of Organoleptic Kefir								
	Kefir grain 2%			Kefir grain 4%			Kefir grain 6%		
	Color	Odor	Flavor	Color	Odor	Flavor	Color	Odor	Flavor
8	1	2.7	3	1	2.7	3	1	2.3	3.3
16	1	1.3	2.7	1.3	2.7	3	1	1.7	3.7
24	1	2	3	1.3	2.3	3.3	1	2	4
32	1.7	2	3	1.7	2	3.7	2	1.3	4
40	2.3	2	3	2.3	2	3.7	2.3	1.3	4
48	3	1.3	4.7	3	1	4.7	2	1	4.7
56	1.7	1	4.7	2.7	1	4.7	2.3	1.3	4.7
64	2.3	1.3	5	2.3	1.3	5	2.3	1.3	5
72	2	1.7	5	2.7	1.3	4.7	2	1.3	5

Note :
Color: Yellow (1), Yellowish white (2), Slightly yellowish white (3), White (4), Very white (5)
Flavor: Very not acidic (1), Not acidic (2), Somewhat acidic (3), Acidic (4), Very acidic (5).
Odor: Very typical of fermented milk (1), Typical of fermented milk (2), Less typical of fermented milk (3), Not typical of fermented milk (4), Foul smelling (5)

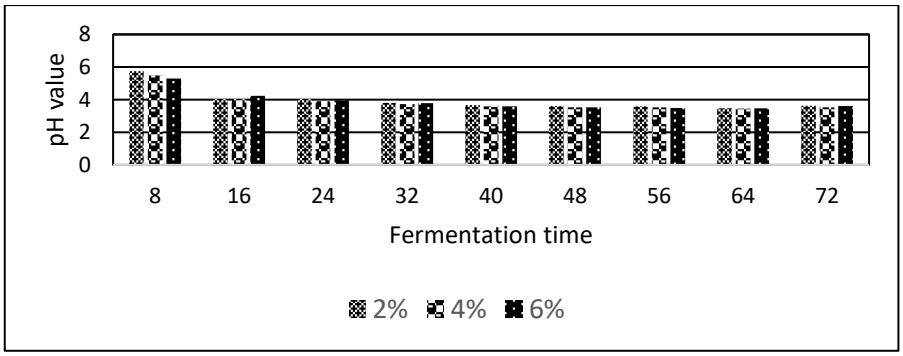

Fig. 2. Effect of starter concentration and fermentation time on the acidity (pH) of sweet corn milk kefir.

The results of pH value of kefir were in accordance with previous research that the fermentation time affects the pH value of kefir [19]. The longer the fermentation time, the lower the pH value as shown in Figure 2. The acid content of sweet corn milk kefir was in a range of 0.323%–2.107%. The highest acid content in 64 hours of fermentation with a 4% starter concentration was 2.107%. According to Codex (2003), the requirement for the value of the lactic acid content of kefir is at least 0.6% [21]. The lactic acid content resulted from 16 to 72 hours of fermentation time met the requirements. Lower pH during fermentation was linked to an increase in lactic acid concentration, which raised the concentration of H^+ ions. The fermentation of milk produced an acidic environment by acid-producing bacteria. Lactic acid bacteria convert lactose to lactic acid during the fermentation process [11]. Kefir's pH tends to stabilize after a 72-hour incubation period, maybe as a result of less readily available substrate.

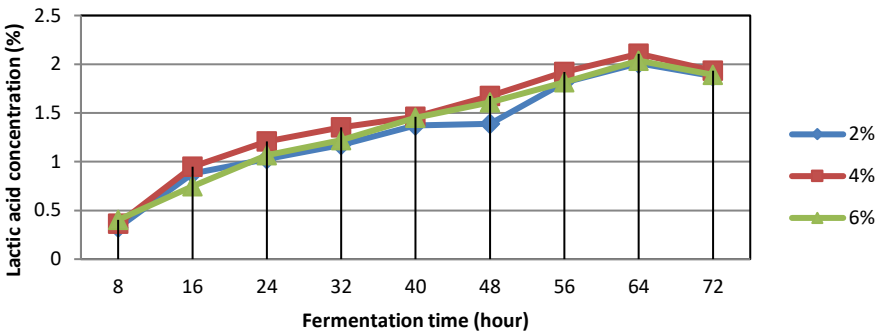

Fig. 3. Effect of starter concentration and fermentation time on lactic acid content of sweet corn milk kefir.

Corn milk kefir exerted antibacterial activity against *V. cholerae* as summarized in Table 2. One-way ANOVA test was conducted to determine the effect of starter concentration and fermentation time of sweet corn milk kefir on the diameter of inhibition zone. The results showed that the diameter of inhibition zone of the negative control was significantly different from those of the concentration of 2%, 4% and 6% (Figure 3). The activity of kefir at all fermentation times was significantly different from the negative control. The antimicrobial properties of kefir could be due to nutrient competition and production of antimicrobial compounds during carbohydrate metabolism, including proteolytic enzymes, organic acids, CO_2, bacteriocins, bioactive peptides, and hydrogen peroxide [12], [25]. The inhibitory activity of kefir may also be due to the presence of lactic acid, acetic acid, propionic acid or the synergistic effect of a number of acidic compounds produced during fermentation [3], [26]. These results suggest that kefir can help prevent gastrointestinal disorders such as diarrhea, one of which is caused by *V. cholerae*. Several studies reported that kefir had inhibitory effect against some pathogenic *Enterobacteriaceae* including *Proteus mirabilis, Micrococcus luteus, Salmonella*, and *E. coli* and also against some fungi such as *Aspergillus fumigatus* and *A. nidulans* [12], [13], [23], [24].

Table 2. The diameter of inhibition zone of sweet corn milk kefir at various starter concentration anf fermentation time againt *V. cholerae*

Time (h)	Diameter of inhibition zone of kefir (mm)		
	Kefir grain 2%	**Kefir grain 4%**	**Kefir grain 6%**
Negative control	-	-	-
8	0.23 ± 0.036*	0.21 ± 0.059*	0.27 ± 0.035*
16	3.76 ± 0.150*	3.80 ± 0.150*	3.61 ± 0.220*
24	3.69 ± 0.201*	3.94 ± 0.012*	3.75 ± 0.144*
32	4.07 ± 0.040*	4.18 ± 0.035*	4.11 ± 0.620*
40	4.15 ± 0.120*	4.17 ± 0.015*	4.16 ± 0.012*
48	7.90 ± 0.040*	8.08 ± 0.040*	7.99 ± 0.032*
56	8.91 ± 0.035*	9.79 ± 0.087*	9.68 ± 0.300*
64	10.08 ± 0.040*	11.02 ± 0.035*	10.54 ± 0.107*
72	10.15 ± 0.032*	10.20 ± 0.026*	10.17 ± 0.030*

*significantly different with negative control ($p<0.000$)

The fermentation time of the kefir had a significant effect on the *V. cholerae* inhibition zone. The longer the fermentation, the more microbes break down glucose into lactic acid and organic acids. However, there is an optimum time due to the limited of energy sources in the form of carbohydrates for lactic acid bacteria. The reduction of nutrients in the medium affects the acceleration of metabolism. The most optimal formula in this research was at a fermentation time of 64 hours and a starter concentration of 4% because it had the lowest pH (acidic), the highest lactic acid content, and the largest inhibition zone against *V. cholerae* with the appearance of a slightly yellowish white color, very sour taste and typical of fermented milk. The results of this study showed a significant correlation between pH value, lactic acid content, and antibacterial activity ($P<0.05$). The correlation of pH vs. antibacterial activity had a negative correlation, which means that the lower the pH value, the higher antibacterial activity. However, the relationship between lactic acid content and antibacterial activity showed a positive correlation (correlation strength of 0.8–1.00), that means the higher the acid content value, the higher the antibacterial effect produced.

Sweet corn milk kefir is a promising alternative for the prevention of some diseases, especially those affecting the gastrointestinal tract. Further studies are needed to conduct in vivo studies to investigate the antimicrobial properties of kefir, particularly in terms of reduced infection rates and severity of symptoms with kefir consumption in animal and human studies.

4 Conclusion

It can be concluded that starter concentration and fermentation time have a significant effect on the properties of kefir. The higher the starter concentration and the longer the fermentation time, the different organoleptic characteristics (color, taste, smell), the decrease in the acidity, the increase in the acid content, and the increase in the inhibition zone against *V. cholerae* bacteria are obtained. The most optimal sweet corn milk kefir formula is at 4% kefir starter concentration with a fermentation time of 64 hours. Further research is needed to improve the properties of sweet corn milk kefir to increase public preference.

The Acknowledgements
Thank you for Pharmaceutical Laboratory for facilitating doing the research.
Author contribution statement
SM: draft preparation, concept & design, manuscript approval; NA: data collection & analysis, manuscript approval.
Conflict of interest
The authors declare no conflict of interest

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