Impact of storage time on stability and antibacterial activity of sweet corn milk kefir

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Abstract. Sweet corn milk can be used as an alternative substrate for kefir with the addition of 10% skim milk and 2% kefir grain. No research has examined the stability of sweet corn milk kefir when stored at 4ºC for long periods of time. The purpose of this study was to determine the effect of 4ºC storage on the stability and antibacterial activity of kefir milk and sweet corn against *Vibrio cholerae*. The sweet corn milk kefir was prepared with the addition of 10% skim milk and 2% kefir grains. It was then stored at 4 oC for one month and subjected to organoleptic test, a stability test, and was also tested for antibacterial activity. The kefir had a yellow color with a pungent, sour taste and a very sour taste in week 4. The average pH ranged from 4.35 to 3.75 and the syneresis ranged from 20.87% to 22.26%. The acid contents obtained ranged from 1.195% to 1.557%. The antibacterial activity was still present after 1 month of storage. The conclusion of this study is that storage at 4°C decreases the pH, increases the viscosity, syneresis, lactic acid content and also the inhibition zone.

1 Introduction

Kefir is a fermented milk that has a distinctive taste similar to yogurt [1]. Conventional kefir is a dairy product that is naturally fermented using a mixed bacterial inoculum and yeast culture found in the kefir grains. Kefir is a probiotic beverage that may have a number of health benefits [2]. Numerous studies have shown an association between regular kefir consumption and a variety of health benefits, and they also support the idea that the probiotic strains in kefir help maintain gastrointestinal health and strengthen the immune system. In addition, kefir can be made using substrates other than dairy milk as the starting point for fermentation, allowing for the creation of novel functional beverages that provide options for consumers [3]. In response to the increasing demand for kefir as a functional food, studies have been conducted on probiotic preparations of kefir using non-dairy kefir, including plant-based kefir with health-promoting properties [4]. This is in line with goal 2 of the Sustainable Development Goals (SDGs), which is to improve nutrition as well as promote sustainable agriculture. If the consumption of food in the community increases, it will be accompanied by the demand for food production.

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One of the vegetables that can be used to make kefir is sweet corn (*Zea mays* L. *saccharata*). Sweet corn has a pleasant taste, contains carbohydrates, protein, vitamins, and is high in sugar but low in fat. Sweet corn has a sweet taste because the sugar content is 5-6%, which is more than regular corn with a sugar content of 2-3% [5], [6]. The nutrients and phytochemicals found in corn include phenolic acids (coumaric acid, ferulic acid, and syringic acid), vitamins A, B, E, and K, minerals (Mg, P, and K), carotenoids and flavonoids (anthocyanins). Numerous studies have examined the benefits of starch found in maize. Consuming a specific quantity of cornstarch can help lower blood sugar and insulin levels while also enhancing intestinal health [5].

Previous studies have shown that corn milk kefir meets kefir criteria with the addition of 10% skim milk and 2% starter concentration. Unfortunately, no research has examined the stability of corn milk kefir when stored at 4ºC for long periods of time in relation to kefir stability and its ability to maintain gastrointestinal health. Long storage of sweet corn milk kefir may result in further fermentation by lactic acid bacteria, which may alter the physical and chemical properties of the kefir.

Previous studies have shown that corn milk kefir meets kefir criteria [7] with the addition of 10% skim milk and 2% starter concentration [6]. Unfortunately, no research has examined the stability of corn milk kefir when stored at 4oC for long periods of time in relation to kefir stability and its ability to maintain gastrointestinal health. Long-term storage of Sweet Corn Milk Kefir may result in further fermentation by lactic acid bacteria, which may change the physical and chemical properties of the kefir. Changes in physical and chemical properties can affect the quality of a probiotic beverage. Therefore, the purpose of this study was to determine how changes in the physical (viscosity, syneresis, and organoleptic)and chemical (pH and lactic acid concentration) properties of sweet corn milk kefir stored at 4ºC.

2 Material and Methods

The materials used in the study were sweet corn, skim milk, kefir grain, *Vibrio cholerae,* Brain Heart Infusion (BHI) media, Mueller Hinton Agar (MHA) media, and NaCl 0.9%.

2.1 Preparation of Sweet Corn Milk Kefir

The first step in making corn milk is to select young, shelled corn kernels weighing 1.5 kg. They are then boiled and mixed with 4.5 liters of water. It was then squeezed and filtered. The corn juice obtained was then added to 10% skim milk powder. It was then pasteurized at 80ºC for 30 minutes [7]. The pasteurized corn milk was then cooled in a glass container until it reached a room temperature of about 18–25°C. Corn milk with a volume of 100 mL was mixed with kefir grains at a concentration of 2% of the total volume of corn milk. Fermentation was carried out for 24 hours at room temperature [8].

2.2 Organoleptical test of Sweet Corn Milk Kefir

The organoleptics including color, odor and taste were observed descriptively.

2.2.1 Color test

Approximately 5 ml of kefir was placed in a test tube with a white background, and then the color of the kefir was observed as: white, slightly yellowish-white, yellowish, and yellow [9].

2.2.2 Odor test

Approximately 5 ml of corn milk kefir was added to the test tube and then smelled. Observations were described as: very dislike, dislike, somewhat like, and like.

2.2.3 Taste test

Sweet corn kefir milk is poured into a spoon and then tasted to feel the taste of the milk. The taste test was observed as: not sour, slightly sour, sour, and very sour [10].

2.3 Acidity (pH) test

Corn milk kefir with a 10% skim milk addition and a 2% kefir starter concentration was tested for acidity level using a pH meter. The electrode was first standardized with buffer 4 and buffer 7 solution standards, and then the electrode was dipped in the sample (kefir) until no significant pH occurred [11].

2.4 Determination of Acid Content

Five grams of corn milk kefir were added to an Erlenmeyer flask, and three drops of 1% pp indicator were added afterward. Subsequently, titration was performed using 0.1 N NaOH while shaking until a consistent pink hue appeared [12].

$$\text{Acid content (\%)} = \frac{\text{ml NaOH x N NaOH x 90.08}}{\text{sample weight (g)x 1000}} \text{ x } 100\ \% \qquad (1)$$

2.5 Viscosity Test

The viscosity test was performde using a Brookfield Viscometer. In this method, a spindle is dipped into the sweet corn milk kefir to be measured for viscosity. The frictional force between the spindle surface and the sweet corn milk kefir determines the viscosity of the liquid. The viscosity is then tested by looking at the number on the viscometer. This viscosity test was performed on day 0, and then the viscosity was tested again after the first week and continued every week for one month.

2.6 Syneresis Test

Syneresis is a stability test to determine whether a liquid layer forms on the surface of the kefir during storage [13]. Centrifuge tubes and samples were weighed and then placed in a centrifuge at 1535 rpm for 20 minutes. The supernantant was separated from the kefir sediment, and then the sediment is weighed in the tube. Syneresis can be calculated using the formula given in Equation (2).

$$\text{Syneresis (\%)} = \frac{A-B}{A} \text{ x } 100\% \qquad (2)$$

A : the initial weight of the sample before centrifugation (gram).
B : the final weight of the sample after centrifugation that has been separated from the supernatant.

2.7 Antibacterial Activity Test

One colony of *V. cholerae* stock culture was streaked on BHI media and then incubated at 37ºC for 18–24 hours [14]. 100 µL of the bacterial culture was put into BHI and incubated at 37ºC for 4–8 hours. Then it was suspended in 0.9% NaCl. After that, the turbidity was adjusted to the 0.5 Mc Farland standard (bacterial concentration of 10^8 CFU/mL). Furthermore, a sterile cotton swab was soaked with inoculum suspension that had been measured for turbidity, and then it was applied to the surface of MHA media until evenly distributed. The MHA was punched using a punch tool, filled with 25 µL the sample (sweet corn milk kefir), and incubated at 37ºC for 20–24 hours. The disc paper contained chloramphenicol as a positive control and sweet corn milk as a negative control. The zone of inhibition formed after incubation was measured using a ruler.

2.8 Data Analysis

The syneresis, viscosity and inhibiiton zone diameter data were tested for normality with the Kolmogorov-Smirnov test and the Levene test to see the homogeneity of the data. If the tested data is normally distributed and homogeneous ($p>0.05$), then proceed with the parametric one-way ANOVA test, followed by the Benferroni posthoc test to determine the difference in data. If the data is not homogeneous or not normally distributed, a non-parametric test with Kruskall-Wallis is performed, followed by the Mann-Whitney test.

3 Results and Discussion

Sweet corn milk kefir that has been made, stored with different storage times, and then carried out organoleptic tests. Organoleptic tests included color, odor, and flavor. Organoleptic tests are commonly used in product quality inspection, process control during processing, and as a method of measuring the quality properties of a product.

Table 1. The results of colour, odour and flavour testing of sweet corn milk kefir with various lengths of 4ºC storage.

Storage time (week)	Color	Odor	Flavor
0	slightly yellowish white	like	slightly acidic
1	yellowish	like	acid
2	yellowish	somewhat like	acid
3	yellow	dislike	very acidic
4	yellow	very dislike	very acidic

Table 1 shows that the color of sweet corn milk kefir become increasingly yellow color along with the length of storage. At week 0, corn milk kefir has a slightly yellowish white color; weeks 1–2 have a yellowish color; and weeks 3 to 4 have a yellow color. The yellow color of corn kefir is due to the carotenoids and riboflavin in corn. Carotenoids and riboflavin are a group of pigments that are yellow, orange, or orange-red pigment, soluble in fat or organic solvents, but insoluble in water [8], [15], [16].

The odor test revealed that panelists liked the taste of sweet corn milk kefir stored at week 0, somewhat liked it at week 2 storage, disliked it at week 3 storage, and strongly disliked it at week 4 storage. The longer the kefir was stored, the more acid and alcohol it produces, so it smells pungent.

The taste test of sweet corn milk kefir was rated as slightly sour at week 0, sour at weeks 1-2, and very sour at weeks 3 to 4. The longer sweet corn milk kefir is stored at 4ºC, the more

sour the taste of sweet corn milk kefir becomes. This is due to an increase in the lactic acid bacteria produced by the corn milk kefir, which also causes the flavor to become more acidic.

The acidity test was conducted to determine if the corn milk kefir had changes in pH during the 4ºC storage period. Figure 1 shows that the longer the storage at 4ºC, the lower the pH and the higher the acidity. The decrease in pH is due to the activity of lactic acid bacteria and yeast from the kefir grains, which convert carbohydrates in the milk into lactic acid and other organic acids. Sweet corn milk kefir produces metabolites in the form of organic acids such as lactic acid, citric acid, and acetic acid during the fermentation process. These organic acids are dissociated acids in the form of H^+ ions. The more acid produced, the more H+ ions are produced, causing the pH to decrease. The measurement of acidity in corn milk kefir from week 0 to week 4 had pH values < 4.65, which is in accordance with Codex 2003 [17]. The total acidity of kefir stored at 4ºC from week 0 to week 4 ranged from 1.1950-1.5571. This level is still in the range of lactic acid content of fermented milk (0.5-2.0%) according to SNI 01-2891-1992.

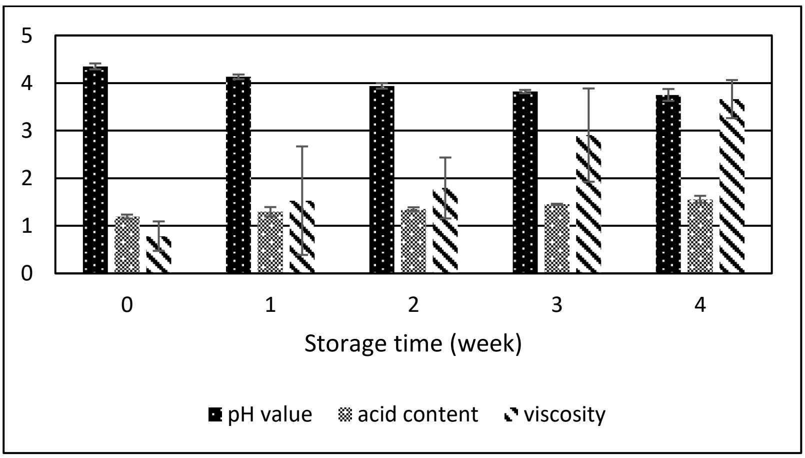

Fig. 1. Average pH value, acid content and viscosity of sweet corn milk kefir at storage time at 4ºC. The longer the storage of kefir, the more the viscosity and the lactic acid content, while the lower the pH value.

Viscosity indicates textural stability, which is directly related to the water-binding capacity of the coagulated milk casein matrix after fermentation.The increase in viscosity in other milk-fermented kefirs is due to lactic acid bacteria producing an exopolysaccharide called kefiran and interacting with milk proteins. Kefiran has the ability to increase the viscosity of fermented milk at low temperature storage [18].

Figure 1 shows that the longer the storage of kefir at 4ºC, the more the viscosity increases. In this study, the viscosity of all samples tended to increase slightly at the end of the storage period. This finding is consistent with previous research who reported that there was an increase in the viscosity of the samples during storage [18]. The increase in viscosity was generally caused by casein from curd produced by lactic acid bacteria during the fermentation process [19]. The statistical test showed a significant difference between week 0 and week 4.

Syneresis measures the degree of retrogradation in kefir storage at lower temperatures and is considered an undesirable property in foods. The values of syneresis for the sweet-corn milk kefir showed significant differences during storage, as summarized in Figure 2. The kefir stored at 4ºC after 4 weeks showed the highest values of syneresis of 22.26%.

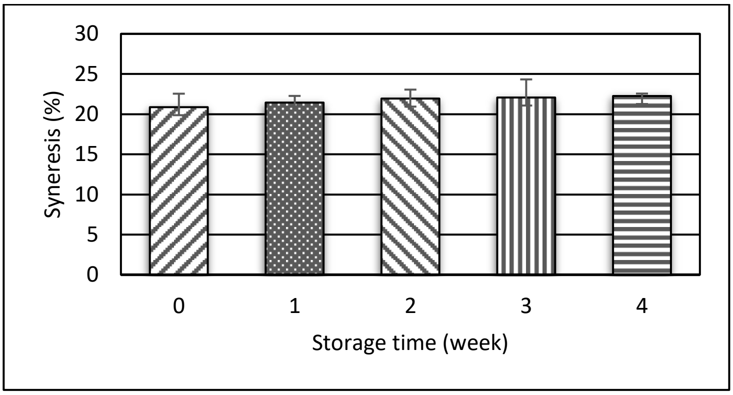

Fig 2. The syneresis of sweet corn milk kefir increased with increasing storage time at 4°C.

Syneresis is a result of the decreased ability of protein tissue to bind water, syneresis is one of the quality parameters of kefir, the higher the syneresis the lower the quality. The occurrence of syneresis may be caused by changes in casein solubility and casein particle shrinkage. Factors that affect kefir syneresis include acidity and pH, as well as water-binding capacity. Longer storage time causes an increase in lactic acid in the kefir, so the acidity of the kefir become higher [20]. High acidity can cause an increase in syneresis because acid causes the hydrolysis of the bond between water and protein. Hydrogen bonds between water and protein molecules are weakened by the acidic environment [15].

Table 2. The diameter of inhibition zone of sweet corn milk kefir at different time of 4°C storage

Storage time (week)	Diameter of inhibiton zone mean ± SD (mm)
0	13.41 ± 0.065*#
1	14.92 ± 0.187*#
2	15.01 ± 0.120*#
3	17.26 ± 0.192*#
4	17.78 ± 0.013*#
Positive control	35.30 ± 0.083*
Negative control	–

Note: diameter was measured including diameter of the well
*Statistical significantly different with negative control
#Statistical significantly different with positive control

V. cholerae can cause foodborne illness when it enters a person. Dehydration, diarrhea, and vomiting are its characteristic. These bacteria can also cause infection and even result in death in humans by causing gastroenteritis and peritonitis [21]. Table 2 shows that the antibacterial activity of sweet corn milk kefir at 4°C storage from week 0 to week 4 showed an increase in antibacterial activity. Statistical results showed a significant difference between the inhibition zone diameer of the negative control and kefir from week 0 to week 4. This indicates that kefir, during storage, still shows the ability to inhibit the pathogenic bacteria *V. cholerae*. However, the bacterial inhibitory activity of kefir is relatively weaker than that of the positive control. The antibacterial activity of kefir tends to increase during storage at 4°C for 1 month due to the possibility of low pH caused by the accumulation of organic acids produced by kefir grains. *V. cholerae* grows well in an alkaline environment (pH 8.0-9.5) and is sensitive to an acidic environment. Kefir with a lower pH produced a larger inhibition zone diameter. This result was in line with other researchs [22], [23].

Our result showed that corn milk kefir during storage at 4ºC for 1 month affected organoleptic (color, odor, and taste), acidity (pH), acid content, viscosity, syneresis, and antibacterial activity. Similarly, a previous study reported that the pH, total acidity, viscosity, and color of carrot-based kefir were significantly affected by the duration of storage at refrigerator temperature [18].The longer the storage time, the corn milk kefir has yellow color with pungent sour smell and very sour taste, lower pH, increasing acid content, increasing viscosity, slightly increasing syneresis, and increasing antibacterial activity.

4 Conclusion

Sweet corn milk kefir in storage for 1 month at 4°C influenced organoleptic (color, odor, and taste), acidity (pH), acid content, viscosity, syneresis, and antibacterial activity. The longer the storage time, sweet corn milk kefir has a yellow color with a pungent sour smell and a very sour taste, a lower pH, increasing acid content, increasing viscosity, slightly increasing syneresis, and increasing antibacterial activity.

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Author contribution statement
Drafting (SM), designing the research (SM), colleting and analysing data (RR), interpreting result (SM), and approving the manuscript (SM, RR)
Conflict of interest
The authors declare no conflict of interest

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