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Original Article

Effect Of Milk and Water Kefir Granules in Sugar Solution

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ABSTRACT

This study investigates the effect of milk and water kefir granules on sugar solution, focusing on their fermentation characteristics and potential applications in the production of functional beverages. Kefir grains, which are symbiotic cultures of bacteria and yeasts (SCOBY), were introduced into sugar solutions to evaluate their fermentation processes, including pH change, sugar consumption, and the production of lactic acid, alcohol, and other metabolites. The two types of kefir granules—milk and water—were compared to determine any differences in fermentation rate, microbial diversity, and the final composition of the fermented solution. Results indicate that both milk and water kefir grains were capable of fermenting sugar solutions, with milk kefir demonstrating a more complex microbial profile and a greater production of lactic acid, while water kefir showed higher ethanol content and a faster fermentation rate. The findings suggest that both kefir types can be used for producing probiotic-rich beverages, with the milk kefir being more suited for dairy-based functional drinks, while water kefir may offer a suitable alternative for non-dairy and vegan applications. The study also explores the broader implications of kefir fermentation in enhancing the nutritional and health-promoting properties of sugar-based substrates.

Keywords: Milk kefir, water kefir, sugar solution, fermentation, probiotics, lactic acid, ethanol, functional beverages.

INTRODUCTION

Effect of Milk and Water Kefir Granules in Sugar Solution

Kefir is a traditional fermented beverage that has been consumed for centuries, primarily known for its probiotic properties and health benefits. It is typically made by fermenting milk or sugar water with specific symbiotic cultures of bacteria and yeast (SCOBY). There are two main types of kefir: milk kefir and water kefir. Both types are produced through the fermentation of a sugar-rich solution, but they differ in the medium used and the microbial communities involved.

- **Milk Kefir**: This is made by fermenting milk (cow, goat, or other animal milk) with milk kefir grains, which consist of a combination of bacteria and yeast.
- **Water Kefir**: This is produced by fermenting a sugar solution (typically water with added sugar) using water kefir grains, which are a different mix of bacteria and yeast than those used in milk kefir.

The **granules** (or grains) in both milk and water kefir are composed of polysaccharides, proteins, and various microorganisms. These microorganisms—lactic acid bacteria, yeasts, and other microbes—carry out the fermentation process, which converts sugars into various end-products, including alcohol, organic acids, and carbon dioxide. This fermentation not only preserves the sugar solution but also imparts various health benefits, including improved digestion and gut health. The focus of this research topic is to explore the effect of milk and water kefir granules in sugar solutions. Specifically, it examines how these kefir granules impact the fermentation process in a sugar solution, including changes in:

1. **Fermentation Rate**: The time it takes for the sugar solution to ferment into kefir.
2. **Microbial Activity**: How the microbial population in the granules influences fermentation dynamics, including microbial growth, metabolic activity, and the production of metabolites like lactic acid and alcohol.
3. **Chemical Changes**: The transformation of sugars into organic acids, alcohol, and other compounds.
4. **Nutritional Changes**: The shift in the nutritional profile of the sugar solution, such as reduced sugar content and increased levels of beneficial compounds like probiotics.
5. **Flavor Development**: The changes in flavor due to the microbial activity, which can range from tart and tangy (due to lactic acid) to slightly alcoholic or effervescent (due to yeast fermentation).

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Understanding how milk and water kefir granules behave in a sugar solution is crucial for optimizing kefir production, both in terms of health benefits and taste. It also contributes to a broader understanding of fermentation science, particularly how different kefir grains interact with varying sugar sources and environmental conditions.

This topic is of interest to researchers in microbiology, food science, and nutrition, and could have implications for the food and beverage industry, where kefir-based products are becoming increasingly popular as functional foods.

MATERIALS AND METHODS

PREPARATION OF WATER KEFIR

Ingredients:

- 2 Tablespoons hydrated Water Kefir Grains
- 1⁄4 cup sugar – organic preferred
- 1 quart of chlorine free water

Requirements:

- Glass Jar
- Mesh strainer
- Cloth with rubber band
- Wooden or plastic spoon and jars

Fig.3.1 WATER KEFIR SAMPLE

Preparation of primary culture(water kefir)

- 100g of sugar was added to the jar.
- Then 500ml water was added to the jar.
- The mixture was stirred thoroughly until the sugar was dissolved.
- 20g of kefir grains was added to the sugar water. Covered with a plastic lid. Kept aside for 24 hours. The kefir has fermented as it smelled tangy. [10]
- A wide mouthed glass container was placed under the strainer , the finished kefir was poured into the strainer, and stirred with a spoon to gently force kefir through the strainer. The grains were separated and collected into a sterile glass container.
- The grains were stored in the fridge.

Fig. 3.2 WATER KEFIR PRIMARY CULTURE

PREPARATION OF MILK KEFIR

Ingredients:

- 1 Tbsp kefir grains
- 4 cups whole cow's milk 960 ml

Requirements:

- Large glass jug (at least 5 cup capacity)
- Wooden or plastic spoon
- Paper towels
- Plastic mesh strainer
- Jar for storing finished kefir

Fig.3.3 MILK KEFIR SAMPLE

Preparation of primary culture (Milk Kefir)

- Initially glass jugs, spoons were steriled 20g of the kefir grains and 500ml of whole milk to a large glass jug was added .
- The jug was covered with plastic lid ,to prevent any bugs or dust from getting in. Fermentation set in a warm, dark spot for about 24 hours[11].
- The kefir has fermented as it was slightly thickened and smelled tangy.

- The kefir has separated into yellowish watery-looking whey.
- A wide mouthed glass container was placed under the strainer , the finished kefir was poured into the strainer and stirred with a spoon to gently force kefir through the strainer .
- The grains were separated and collected into a sterile glass container.

The grains were stored in the fridge

Fig.3.4 MILK KEFIR PRIMARY CULTURE

ESTIMATION OF SUGAR BY UV-VISIBLE SPECTROSCOPY

INGREDIENTS :

- Sulphur free sugar
- Sodium hydroxide
- Sodium potassium tatarate
- Distilled water
- Ethanol

REQUIREMENTS

- UV-Visible spectrophotometer
- Volumetric flask
- Pipette
- Beaker

UV –VISIBLE SPECTROPHOTOMETER

PREPARATION OF SOLUTIONS

Glucose solution (100mg %) : 100 mg of standard sulphur free sugar is dissolved in 100ml of distilled water.

2N NaOH : 8g of sodium hydroxide pellets is dissolved in 100 ml of distilled water to form 2N NaOH.

PREPARATION OF DNSA SOLUTION :

- 1g of DNSA (di-nitro salicylic acid) is dissolved in 20 ml of 2N NAOH.
- 50 ml distilled water was added into the flask .
- 30g of Rochelle's salt was added and was dissolved.
- Volume was madeup to 100 ml with distilled water .It was then filtered in and stored amber coloured bottle.

PREPARATION OF WATER KEFIR SAMPLE:

- 100mg of sugar was added to the jar.
- Then 100ml water was added to the jar.
- The mixture was stirred thoroughly until the sugar was dissolved.
- 300mg of kefir grains was added to the sugar water. Covered with a plastic lid. Kept aside for 24 hours
- The kefir has fermented smelled tangy. [10]
- A wide mouthed glass container was placed under the strainer , the finished kefir was poured into the strainer, and stirred with a spoon to gently force kefir through the strainer .
- The grains were separated and collected into a sterile glass container.
- The grains were stored in the fridge .

PROCEDURE FOR WATER KEFIR :

- 0,2,4,6,8ml of above sample solution was pippetted out into each 25 ml volumetric flask.
- 3 ml of 2N NaOH was added to all flasks.
- 3 ml DNSA solution was added to all flasks.
- The flasks were made up to 25ml .
- Flasks were kept in boiling water for 15 minutes.
- The flasks were kept under the running water and the absorbance was measured at 540 nm.

PREPARATION OF MILK KEFIR SAMPLE :

- 300mg of the kefir grains and 100ml of whole milk to a large glass jar was added .
- The jug was covered with plastic lid ,to prevent any bugs or dust from getting in. Fermentation set in a warm, dark spot for about 24 hours[11].
- The kefir has fermented as it was slightly thickened and it smelled fermented.
- The kefir has separated into yellowish watery-looking whey.
- A wide mouthed glass container was placed under the strainer , the finished kefir was poured into the strainer, and stirred with a spoon to gently force kefir through the strainer .
- The grains were separated and collected into a sterile glass container.
- The grains were stored in the fridge.

PROCEDURE FOR MILK KEFIR

- 0, 2,4,6,8ml of above sample solution was pippetted out into 25 ml volumetric flask.
- Add 3 ml of ethanol to all flasks.
- The flasks were made up to 25 ml using water.
- Stored in the fridge for 1 hour.
- The flasks were brought to normal temperature and the absorbance is measured at 208 nm.

RESULTS AND DISCUSSIONS:

Milk and Water kefir beverages were prepared. The absorbance of the prepared drinks were estimated and compared with the standard sugar water and milk using UV-VISIBLE SPECTROPHOTOMETER. We have observed reduction in the sugar concentration of kefir fermented beverages .Kefir has a tangy flavor and a consistency similar to drinkable yogurt. Due to the fermentation process, kefir tasted slightly carbonated. We have observed the biomass of kefir grains. Initially we have taken 20g of water and milk kefir grains. For 1st culture of water kefir the grains weight increased gradually to 28g for 2nd culture it increased up to 31g & for 3rd culture it increased to 34g. Later the mass of the kefir grains were decreased. Same as for milk kefir, 20g lo kefir grains were taken. For 1st culture it increases upto 26g for 2nd culture it increases up to 29g & for 3rd culture it increases up to 30g after the 3rd culture the weight of the kefir grains were decreased.

ESTIMATION OF ABSORBANCE OF SUGAR CONCENTRATION IN WATER KEFIR

Standard

Table 5.1 Results of absorbance of standard

s.no	Concentration (µg/ml)	Absorbance (nm) (standard)
1.	0	0
2.	80	0.220
3.	160	0.426
4.	240	0.650
5.	320	0.854

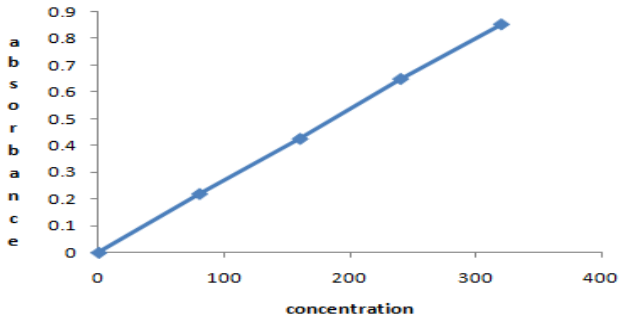

Fig no.4.1 Calibration curve for standard.

Day -1

Table 5.2 Results of absorbance of water kefir on day -1

s.no	Concentration (µg/ml)	Absorbance (nm) (standard)	Absorbance (nm) (sample)
1.	0	0	0
2.	80	0.220	0.220
3.	160	0.426	0.421
4.	240	0.650	0.622
5.	320	0.854	0.849

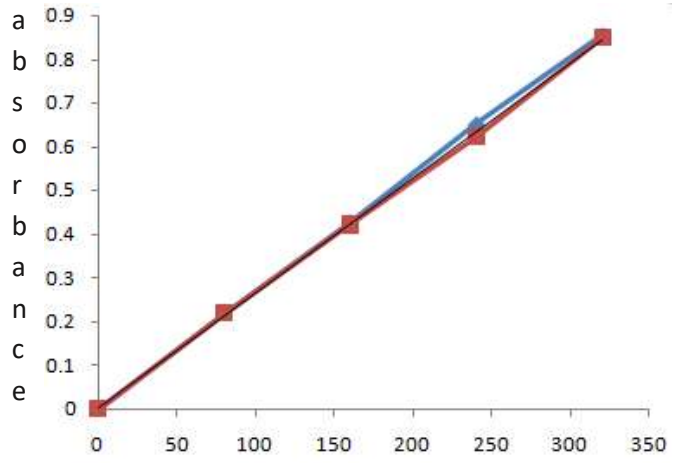

Fig no.4.2 Calibration curve for standard vs sample day 1

Day -2

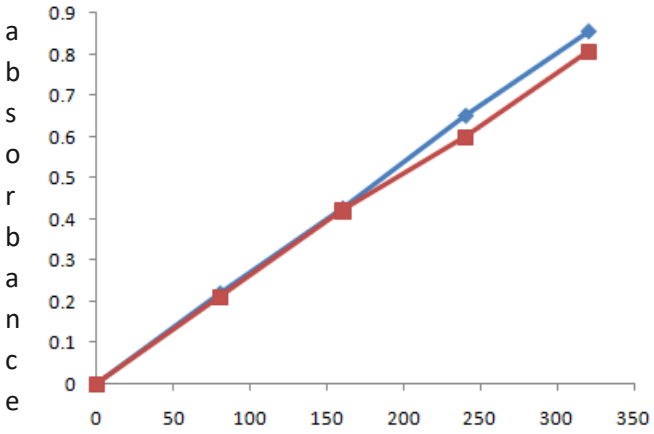

Fig no.4.3 Calibration curve for standard vs sample day 2

Table 5.3 Results of absorbance of water kefir only day-2

s.no	Concentration (µg/ml)	Absorbance in nm (standard)	Absorbance (nm) (sample)
1.	0	0	0
2.	80	0.220	0.150
3.	160	0.426	0.353
4.	240	0.650	0.499
5.	320	0.854	0.687

Day -3

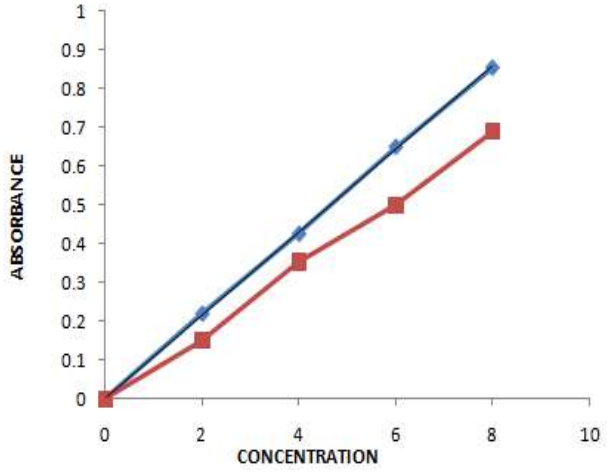

Fig no.4.3 Calibration curve for standard vs sample day 3

Table 5.3 Results of absorbance of water kefir only day-3

s.no	Concentration (µg/ml)	Absorbance (nm) (standard)
1.	0	0
2.	80	0.195
3.	160	0.369
4.	240	0.561
5.	320	0.722

ESTIMATION OF ABSORBANCE OF SUGAR CONCENTRATION IN MILK KEFIR

Standard

Table 5.5 Results of absorbance of standard lactose

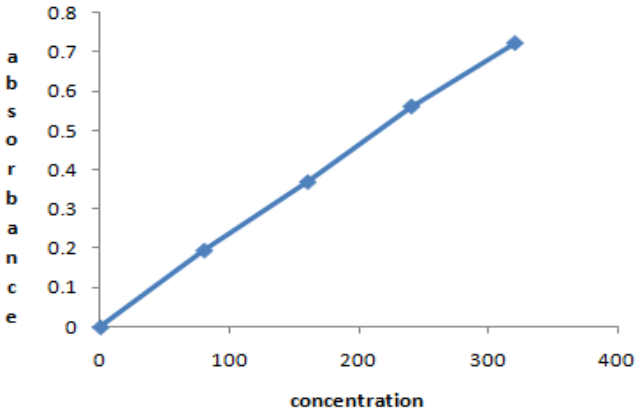

Fig no.4.4 Calibration curve for milk standard

Day -1

Table 5.6 Results of absorbance of milk kefir on day-1

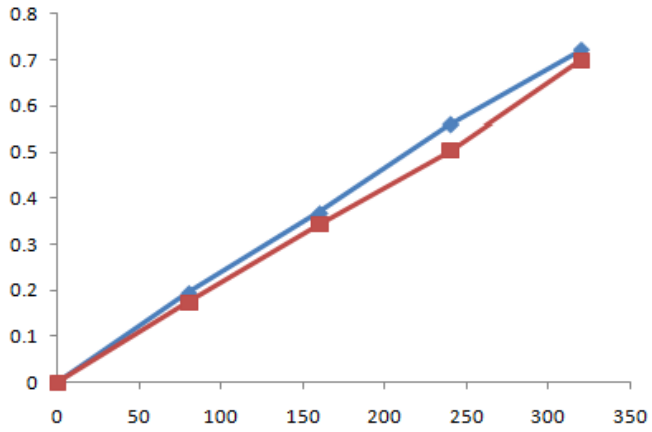

Fig no.4.5 Calibration curve for milk standard vs sample day1

Day -2

Table 5.7 Results of absorbance of milk kefir on day -2

s.no	Concentration (µg/ml)	Absorbance in nm (standard)	Absorbance (nm) (sample)
1.	0	0	0
2.	80	0.195	0.174
3.	160	0.369	0.344
4.	240	0.561	0.505
5.	360	0.722	0.699

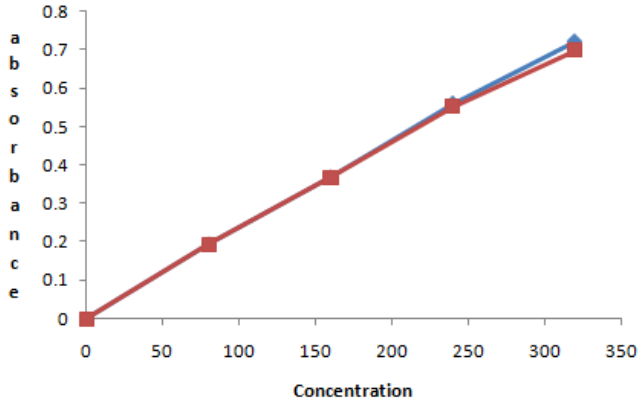

Fig no.4.6 Calibration curve for milk standard vs sample day2

Day -3

Table 5.7 Results of absorbance of milk kefir on day-3

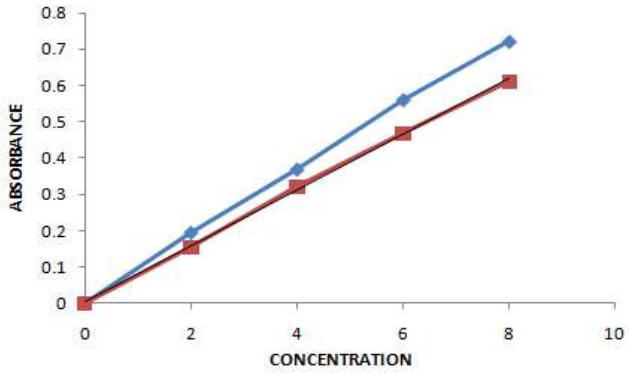

Fig no.4.4 Calibration curve for milk standard vs sample day3

From the above graphs we can conclude that fermented kefir beverages have shown that there is reduction in sugar concentration when compared with standard.

SUMMARY AND CONCLUSION

Kefir grains have shown the ability to reduce sugar concentration during fermentation, with studies demonstrating a significant decrease in sugar content, such as a reduction of approximately 40% after 96 hours of fermentation. The conversion of sucrose to glucose and fructose during fermentation contributes to this reduction, with variations observed in sugar content when kefir grains interact with different substrates like organic brown sugar The conversion of sucrose to simpler sugars like glucose and fructose, along with the interaction between kefir grains and various substrates, influences the sugar content in the final product. The fermentation process occurs when milk is combined with kefir grains and left to ferment at room temperature for several days. During this process, the kefir grains break down the lactose in the milk, creating lactic acid, alcohol, and acetic acid. The water kefir grains utilize the sugar in the solution to produce lactic acid, carbon dioxide, and a small amount of ethanol. This process involves the breakdown of sucrose into simpler compounds like glucose and fructose by the microorganisms present in the grains.

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