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Physicochemical and microbiological analysis of the probiotic Kefir beverage

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Abstract

The best prebiotics to increase the amount of probiotic bacteria in the gastrointestinal tract have been the subject of extensive research. The goal of the current study was to ascertain how adding ragi flour and barnyard flour as prebiotics would affect the characteristics of various probiotic strains in kefir as well as their ability to survive cold storage. The addition of ragi flour and barnyard flour did not influence the chemical parameters but the viability of probiotic strains increased or remained unchanged during storage. In addition to that the biochemical properties are also enhanced due to the presence of Prebiotics. The control sample and T1, T2, and T3 experimental samples were developed and analyzed their proximate composition. The results indicated that the control sample had 11.73 % of protein. The protein content of kefir beverages (T1, T2 and T3) varied from 12.63 % to 13.33 %. The control sample had 2.79 % of fat. The fat content of experimental products ranged from 2.43 % to 2.87%. The T3 sample had the highest fat content compared to the rest of the formulations. Dietary fiber was very low in the control sample and it ranged from 2.75 % to 3.08 % in experimental products. The control sample exhibited the highest carbohydrate content 85.47 %, and the experimental products exhibited a carbohydrate content ranging from 81.12 % to 82.06 %. The proximate values were significantly different ($P < 0.05$). The control and experimental products were subjected to sensory analysis, and results indicated that the experimental product T3 got the highest score for appearance compared to T1 and T2. The sensory score for flavor for T3 (8.24 ± 0.55) and control (8.72 ± 0.37) was more consistent compared to the remaining two products. The taste, texture, and overall acceptability of T3 was more consistent with control samples compared to T1 and T3. The experimental product T3 was superior in terms of appearance, flavor, taste, consistency, and overall acceptability compared to other formulations. The physiochemical analysis showed that kefir contains a good amount of phenolic compounds as well and it also has great antioxidant activity this factor makes the kefir a great probiotic drink with a lot of health benefits.

Keywords barnyard flour, kefir beverage, kefir grains, ragi flour

Introduction

Kefir is one of the functional foods, a popular, natural probiotic beverage that acts as an effective tool for the delivery of probiotic microorganisms and accompanies a critical function in the ecological stability and the therapeutic activity of the host. It is a fermented beverage made from cultures that taste like yoghurt. Probiotics are live bacterial biomodulators known to prevent or treat disruptions in the gut microbiota and improve the host's health. Food supplements containing probiotics and symbiotics, which are safe and inexpensively produced and are known to enhance gut homeostasis, are already being consumed. The starter is a blend of milk, bacteria, proteins, and yeast. It has a tart, creamy flavor and is loaded with various probiotic health benefits.

Kefir has many therapeutic and functional benefits, such as boosting the immune system, reducing cholesterol, easing the symptoms of lactose intolerance, and having anti-mutagenic and anti-carcinogenic properties. Numerous investigations have demonstrated the anticarcinogenic, antimicrobial, and synbiotic properties [1-2].

It is well known that dietary fiber has a functional mechanism of action that improves stool transit time, and increases stool bulking and water retention. Additional advantages of certain plant-based dietary fibers include modulation of the gut microbiota, antioxidant and anti-inflammatory properties, and support for metabolic health. Clinical evidence supports the use of psyllium, inulin, arabinogalactan, partially hydrolyzed guar gum (PHGG), and acacia gum as specific nutritional threads to support improved functional bowel health. Pectin oligosaccharides and slippery elm (Ulmus rubra) have been used in traditional medicine to support gastrointestinal health, though they are not specifically recommended for constipation [3].

There is a growing global trend towards the consumption of fermented foods that have health-promoting properties. Escalating worry over nutrition and medical conditions has resulted in the creation of more advantageous and sustainable food items [4].

The aforementioned facts the research study was designed to prepare the functional kefir beverage with the addition of ragi flour and barnyard flour and we study the preparation of kefir beverage and its sensory, proximate, and microbial analysis.

Methodology

Collection of raw material

Whole milk, skimmed milk powder, Ragi flour, and barnyard flour were used in this experiment. These ingredients were purchased from the local markets of reliable stores. Freeze-dried kefir starter cultures were purchased from the Health from Home Loft online store. The kefir starter culture was composed of *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Saccharomyces* according to the manufacturer.

Preparation of Kefir beverage

The fermented Kefir beverage was prepared with the addition of ragi flour and barnyard flour according to the formulation (Figure 1, Table 1). The control and samples T1, T2, and T3 were developed and stored at refrigeration conditions until analyzed. The Kefir beverage was prepared by using pasteurized milk. The skim milk powder was added to the pasteurized milk and mixed properly. The ragi flour was added to the mixture along with the inoculation of the starter culture to prepare T1 experimental product in a ratio of 25:25.

T2 experimental product was prepared by adding 25 % of barnyard flour and 25 % of starter culture. T3 product was prepared by adding starter culture, ragi flour, and barnyard flour in a ratio of 25:13:12. All the prepared samples were subjected to fermentation to obtain the kefir beverage and stored under refrigeration conditions until used for further analysis.

Physico-chemical properties

Association of official analytical chemists procedures were followed for the estimation of physical properties and proximate composition of the prepared functional kefir beverage [5].

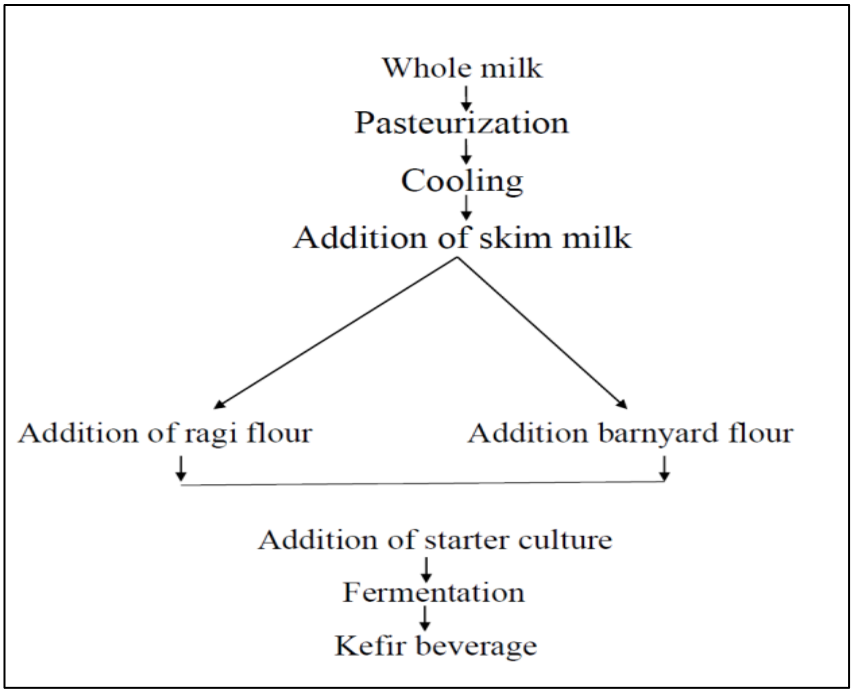

Figure 1. Schematic representation of preparation of kefir beverage

Table 1. Physicochemical parameters

Ingredients	Control	T1	T2	T3
Whole milk	50 %	25 %	25 %	25 %
SMP	25 %	25 %	25 %	25 %
Kefir starter grains	25 %	25 %	25 %	25 %
Ragi flour	-	25 %	-	13 %
Barnyard flour	-	-	-25 %	12 %

Sensory evaluation

The prepared kefir samples were analyzed for determination using a semi-trained panel (45) via 9 9-point hedonic scale [5].

Determination of antioxidant activity

The scavenging effect of kefir samples was determined with the DPPH method using vitamin C as a control. The triplicate values were taken [6].

$$\% \text{ Scavenging effect} = (1- a/b) \times 100$$

Where a and b were the absorption at 517 nm of sample and control.

Determination of antimicrobial activity

The antibiotic activity of the samples was estimated using the diffusion method [7].

Microbial analysis

Analysis of Total plate count (TPC), Yeast, and mould content was performed at the following time intervals: First day-post-production and 15th and 30th day post-production at room temperature (27±3°C), refrigerated temperature (less than 5°C) and frozen conditions (-18°C) [8].

Statistical analysis

The data of triplicate values were subjected for statistical analysis using one way analysis of variance and Duncan's multiple comparison test.

Results and Discussion

Preparation of Kefir beverage

The method followed for the preparation of Kefir beverages is presented in Figure 1. The texture and color of the prepared samples (T1, T2, and T3) fermented Kefir beverages has been shown in Figure 2.

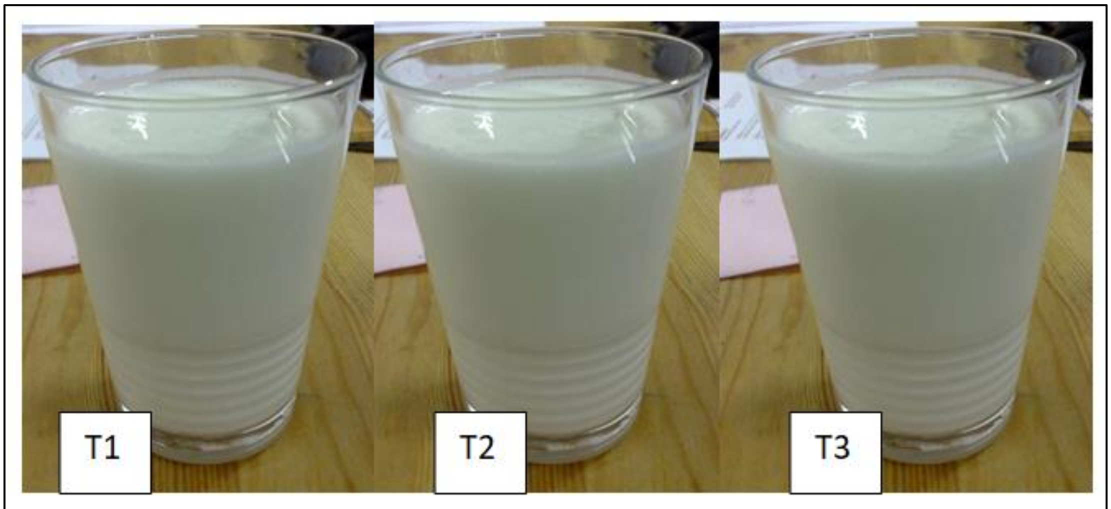

Figure 2. Texture and color of the prepared samples (T1, T2, T3) fermented Kefir beverages

Physico-chemical properties

The proximate compositions of different samples of Kefir beverage are presented in Table 2. The results indicated that the control sample had 11.73 % of protein. The protein content of experimental products were ranged from 12.63 % to 13.33%. The protein values of T1 and T3 were not significantly different, but the remaining samples were significantly different ($p < 0.05$) according to the Duncan multiple comparison test.

Table 2. Proximate composition of different samples of Kefir beverage

Composition (%)	Control	T1	T2	T3
Protein	11.73 ± 0.28^{b}	13.33 ± 0.76^{a}	12.63 ± 0.32^{ab}	13.22 ± 0.78^{a}
Fat	2.79 ± 0.36^{a}	2.46 ± 0.50^{a}	2.43 ± 0.50^{a}	2.87 ± 0.56^{a}
Dietary fibre	0.00 ± 0.00^{b}	3.08 ± 0.38^{a}	2.88 ± 0.39^{a}	2.75 ± 0.22^{a}
Carbohydrate*	85.47 ± 0.44^{a}	81.12 ± 1.27^{b}	82.06 ± 0.88^{b}	81.15 ± 1.01^{b}

Values were expressed as mean ± sd. The values in the same row with different superscript letters are significantly different ($p < 0.05$) as per Duncan's multiple comparison test. * calculated values.

The control sample had 2.79 % of fat. The fat content of experimental products ranged from 2.43 % to 2.87%. The T3 sample had the highest fat content compared to the rest of the formulations. Dietary fiber was zero in control samples and it ranged from 2.75% to 3.08 % in experimental products. The control sample exhibited the highest carbohydrate content 85.47 %, and the experimental products exhibited the carbohydrate content ranging from 81.12 % to 82.06% (Table 2).

The carbohydrate content of experimental products was not significantly different ($p < 0.05$) as per Duncan's multiple comparison test. These values were more consistent with control samples and similar results were reported by Ganatsios et al., [1] for kefir beverages.

Sensory analysis

The experimental product T3 got the highest score for appearance compared to T1 and T2 (Table 3). It may be explained by the reasoned that the formulation of T3 and its ingredients. The sensory score for flavor for T3 (8.24 ± 0.55) and control (8.72 ± 0.37) was more consistent compared to the remaining two products. The taste, texture, and overall acceptability of T3 was more consistent with control samples compared to T1 and T3 (Table 3). It may be explained by the reasoned that the T3 product formulation is composed of the correct percentage of ingredients.

Table 3. Sensory analysis of Kefir beverage

Sample	Appearance	Flavour	Taste	Texture/ consistency	Overall acceptability
Control	8.82 ± 0.40^{a}	8.72 ± 0.37^{a}	8.74 ± 0.43^{a}	8.78 ± 0.42^{a}	8.80 ± 0.38^{a}
T1	6.76 ± 0.48^{c}	7.80 ± 0.55d	6.98 ± 0.71d	6.55 ± 0.63c	6.68 ± 0.74c
T2	8.24 ± 0.44^{b}	8.14 ± 0.62^{c}	7.93 ± 0.76^{c}	7.22 ± 0.40^{b}	7.35 ± 0.48^{b}
T3	8.80 ± 0.39^{a}	8.24 ± 0.55^{b}	8.39 ± 0.65^{b}	8.66 ± 0.48^{a}	8.68 ± 0.47^{a}

Values were expressed as mean ± sd (n = 45). The values in the same column with different superscript letters are significantly different ($p < 0.05$) as per Duncan's multiple comparison test.

Determination of antioxidant activity

The barnyard kefir T2 exhibited the maximum antioxidant value of 1.718 µg/ml compared with other kefir samples and the T3 (Ragi and barnyard mixed) sample exhibited 0.989 µg/ml.

Determination of antimicrobial activity

The antibiotic activity of kefir milk (ragi kefir and barnyard kefir) was assessed using the well diffusion method (Figure 3).

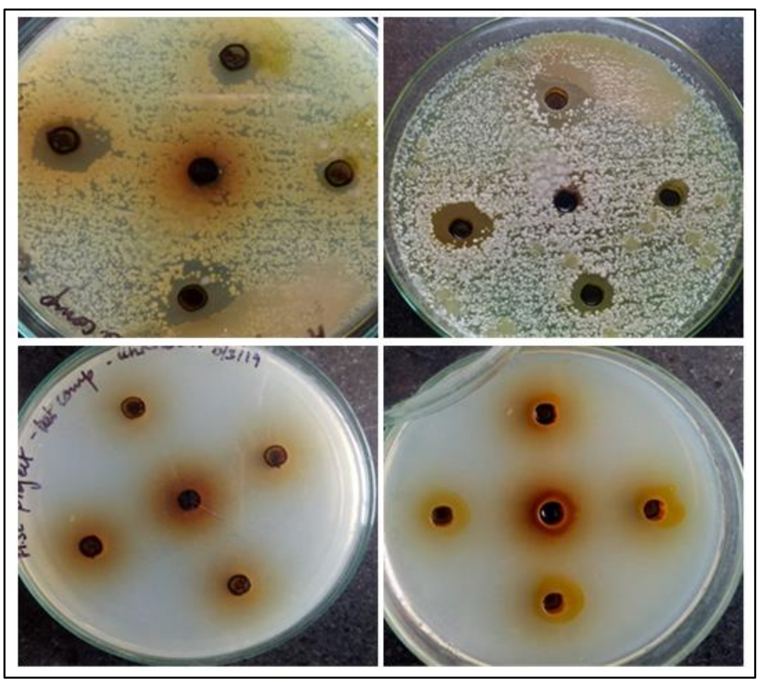

Figure 3. Antimicrobial activity of kefir beverages

The organism *E. coli* showed maximum susceptibility to kefir (13 mm) klebsiella pneumonia (11mm) Enterococcus faecalis showed maximum zone of inhibition of (9 mm) Staphylococcus aureus (16 mm) and Proteus. spp showed a maximum zone of inhibition with (10 mm). Candida albicans showed a maximum of (7 mm) inhibition. All activity tests were performed in triplicate.

Shelf life studies

Microbial analysis results were presented in Tables 4 and 5. The results indicated that the TPC and total yeast and mould count were nil at freezing conditions for 30 days storage period. These results indicated that the optimum storage conditions for kefir beverages were refrigeration and freezing for 30 days.

Table 4. Total Plate count

Storage conditions	Period	TPC (CFU/g)			
		Control	T1	T2	T3
Refrigerated temperature (less than 5°C)	0th day	0	0	0	0
	15th Day	-	-	-	-
	30th Day	-	-	-	-
Frozen conditions (-18°C)	0th day	0	0	0	0
	15th Day	0	0	0	0
	30th Day	0	0	0	0
Ambient conditions (27±3°C)	0 hour	1 X 101	0	0	0
	24 hour	6 X 103	8 X 102	7 X 102	7 X 102
	48 hour	16 X 106	22X 104	21X 104	20 X 105

Table 5. Yeast and mould count

Storage conditions	Period	(CFU/g)			
		Control	T1	T2	T3
Refrigerated temperature (less than 5°C)	0th day	0	0	0	0
	15th Day	0	0	0	0
	30th Day	0	0	0	0
Frozen conditions (-18°C)	0th day	0	0	0	0
	15th Day	0	0	0	0
	30th Day	0	0	0	0
Ambient conditions (27±3°C)	0 hour	0	0	0	0
	24 hour	7	0	0	0
	48 hour	9 X 102	3	2	4

Conclusion

The Kefir beverage was prepared with different formulations and subjected for sensory analysis, and proximate and microbial analysis. The T3 was superior in terms of sensorial parameters compared to the rest of the formulations. The results indicated that the control sample had 11.73 % of protein. The protein content of T1, T2 and T3 varied from 12.63 % to 13.33 %. The control sample had 2.79 % of fat. The fat content of experimental products ranged from 2.43 % to 2.87%. The T3 sample had the highest fat content compared to the rest of the formulations. Dietary fiber was zero in the control sample and it ranged from 2.75 % to 3.08 % in experimental products. The control sample exhibited the highest carbohydrate content 85.47 %, and the experimental products exhibited a carbohydrate content ranging from 81.12 % to 82.06 %. The microbial analysis results indicated that the optimum storage conditions for kefir beverages were freezing and refrigeration conditions for 30 days. The physiochemical analysis showed that kefir contains a good amount of phenolic compounds as well and it also has great antioxidant activity this factor makes the kefir a great probiotic drink with a lot of health benefits. This study exhibits that kefir is a potential antioxidant that interacts with a wide range of species directly responsible for oxidative damage. The antioxidative activity of kefir may be attributed to their proton-donating ability to reduce power and SOD-like activity, as evidenced through DPPH activity. Therefore, kefirs are a potential beverage for the role of useful and natural antioxidant supplements in the human diet. Probiotics, found in kefir, have been demonstrated to possess antimicrobial and carcinogenic qualities among other useful attributes. Reduced cholesterol and improved lactose intolerance are two ways it benefits people's health. Standard ethanol was used for the antimicrobial activity tests because it is an antimicrobial agent produced by yeast in kefir. However, the ethanol solution did not affect the growth of any test strains. The probiotic strains' viability during storage and the final product's sensory qualities were both enhanced by the addition of Ragi and Barnyard millet flour along with probiotic strains.

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