

Chemical and Rheological Characterization of Functional Kefir Supplemented with Basil Seed Mucilage Extract

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Abstract

Introduction: There is a growing demand for different dietary interventions focused on improving gut health of consumers. Fermented dairy products with added plant-based additives are currently gaining more attention due to their nutritional and therapeutic potential. **Objectives:** This study examined the chemical and rheological properties of kefir drink supplemented with basil seed mucilage (KBS). **Method:** To formulate the drink, cow milk was inoculated with kefir grains and basil seed mucilage extract (BSME) at 25 °C for 24 h. The bioactive compounds were quantified using GC-MS and rheological properties were assessed using digital viscometer under varying shear rates. **Results:** GC-MS analysis revealed diverse range of bioactive compounds in KBS, including essential fatty acids, esters, furans, nitrite, amino acid derivatives and polysaccharides, all of which are known to have antimicrobial, antioxidant, antiproliferative, and anti-inflammatory properties. Rheological measurements indicated that both KBS and cow milk kefir (control) exhibited pseudoplastic non-Newtonian (shear-thinning) behavior ($n < 1$). However, the KBS demonstrated significantly higher apparent viscosity ($441 \text{ mPa}\cdot\text{s}$ at 27.9 s^{-1}) and consistency coefficient ($K = 3.294$) than cow milk kefir ($p < 0.0001$), attributed to the thickening effect of BSM. **Conclusions:** These findings suggest that BSME modifies texture and incorporates unique bioactives, thereby enhancing the functional and sensory attributes of KBS. This research facilitates valuable awareness on formulations of health-promoting drinks that can be marketed for their nutritional benefits.

Keywords: Rheological, GC-MS, Kefir, Basil Seed, Synbiotics, Probiotics

1. Introduction

The increasing prevalence of lifestyle-related diseases, including obesity, diabetes, and gastrointestinal disorders, has led to trend shift towards healthy food products with high nutritional value ¹. Synbiotics are products that integrate both probiotics and prebiotics and are emerging as promising solutions to enhance gut health and overall well-being. Probiotics are bacteria that when administered in appropriate quantities, demonstrate the efficacy in improving gut microbiota. Probiotics prevent pathogen colonization by competitive exclusion, adhesion to the mucosal surface, and the secretion of antimicrobial compounds such as bacteriocins, organic acids, and hydrogen peroxide. They produce short chain fatty acids that maintain energy homeostasis and regulate tissue functions. Probiotics enhance cell adhesion, modulate immune system activity, and their metabolites interact with the brain-gut axis to influence behaviour ². However, prebiotics are the class of plant derived polysaccharides that are, non-digestible in nature which stimulate the growth and activity of beneficial microorganisms ^{3, 4}.

Kefir is a traditional probiotic rich fermented milk-based drink, produced using kefir grains⁵. Kefir grains appear similar to cauliflower florets in cream color and consist of diverse microbial communities, including lactic acid bacteria, acetic acid bacteria and yeast. Various factors such as type of milk, specific variety of kefir grains, and processing techniques can significantly influence the sensory and chemical properties of kefir ⁶⁻⁸. Several research studies have demonstrated the therapeutic potential of kefir, including anti-inflammatory effects, modulation of gut microbiota, and protective benefits against gastrointestinal diseases ^{9, 10}.

Bioactive compounds present in kefir are exopolysaccharides, organic acids, lactic acids and peptides, which contribute to their therapeutic potential ^{5, 11}. Additionally, ethyl alcohol and acetaldehyde present in kefir contribute to distinctive flavor of the drink ⁶. Milk gel formation is a crucial rheological property of fermented dairy products¹². Rheological properties help to determine the interactions and structural composition of kefir formulation. Multiple attributes, including incubation, temperature, storage time, additives and kefir grains composition may change the rheology of kefir, which plays a significant role in acceptance among consumers ^{12, 13}.

Recent research underscores the synergy between the integration of plant-based additives into probiotic rich food products, which improves nutritional, functional, and therapeutic profile. Plant based additives such as fruit pulps, seed mucilage, and herbal extracts enhance health beneficial compounds, including antioxidants, phenols, dietary fibers, and short chain fatty acids (SFAs)¹⁴. Studies have shown that probiotic rich fermented milk enriched with plant based derivatives improved irritable bowel syndrome symptoms¹⁵, boosted immune response¹⁶, lowered type 2 diabetes and LDL cholesterol levels¹⁷. Despite the added health benefits, plant-based additives may compromise the microbial, texture and sensory properties ¹⁴. Thus, comprehensive research is important for strategic formulation of probiotic-rich products.

Basil seed (*Ocimum basilicum* L.) is a well-known medicinal plant that is, rich in dietary fiber, including fructooligosaccharides and characterised as a prebiotic which promote the production of SFAs that are beneficial for gut health^{18, 19}. Basil seeds contain hydrophilic polysaccharide-based mucilage content composed of arabinose, xylose, rhamnose and galacturonic acid. The basil seed mucilage (BSM) exhibits the high water binding capacity that swells in water to create gel-like matrix^{18, 20}. BSM used in this study was extracted from *Ocimum basilicum* L. seeds, which are widely recognized and approved for use as food ingredients which are widely recognized and approved for use as food ingredients²⁰⁻²². The phenolic compounds such as rosmarinic acid, caffeic acid, and flavonoids present in the BSM helps to mitigate oxidative stress, and improve anti-inflammatory efficiency²³. The BSM demonstrates high viscosity at low shear rates, which decrease under applied stress. In previous research study, the addition of 0.5% BSM to low-fat milk protein increased the viscosity to 35% while maintaining shear thinning behavior²⁴. The incorporation of BSM is a valuable functional additive in fermented foods, providing multifunctional benefits. It improves texture by acting as a natural thickener and stabilizer, reduces whey separations, and enhances consumer acceptance. Its high water-holding capacity and thermal stability improve product consistency and extend shelf life under fermentation conditions. Additionally, basil seed mucilage contains soluble fiber and bioactive compounds, providing prebiotic effects that support probiotic viability and gut health²⁴⁻²⁶.

Although, plant based rich probiotic products shows the promising nutritional and therapeutic potential, there is still a market gap for these products. Such formulations not only exhibit improved health benefits, but also meet the interest of consumers for functional products. Combination of kefir with added bioactive rich BSM creates a synbiotic synergy, which have probiotic richness from kefir and prebiotic properties from BSM. The aim of this research study was to investigate comprehensive chemical composition in functional kefir drink supplemented with BSM (KBS). Further, this study also analysed the rheological properties (torque, shear stress, apparent viscosity) of the KBS in comparison to traditional cow milk kefir at each shear rates and storage duration.

2. Methods

2.1 Ingredients

Milk kefir grains used in this study were procured from the Culture Market, Sri Dhanwantari Probiotics Pvt. Ltd. (Tamil Nadu, India). Kefir grains were immersed in cow milk, which was changed every 3-5 days and stored in refrigerator at 4-8 °C to maintain the viability of the kefir grains. Basil seeds were procured from HW Wellness Solutions Pvt. Ltd. (Maharashtra, India). Commercially available pasteurized full-cream cow's milk was used in this study.

2.2 Preparation of Basil Seed Mucilage Extract (BSME)

Seeds were immersed in mineral water at seed-to-water ratio of 1:65 (w/v) and kept in water bath, at 65°C and 70 rpm for 15 min. Further, swelled basil seeds were filtered using a double mesh strainer and processed in grinder mixer at low speed for 1 min. The resultant mixture was then centrifuged at 25°C and 15,000 rpm for 10 min. The supernatant was subsequently stored at 4–8°C for future fermentation purposes.

2.3 Formulation of functional kefir drink supplemented with BSME (KBS)

To prepare the KBS, the previous established protocol by Chadha et. al., 2024²⁷ with slight modifications was utilised. Commercially available pasteurised full-cream cow milk was inoculated with 3% (w/v) kefir grains, along with 0.3% (w/v) BSME, and incubated for 24 h at 25 °C. Following fermentation, kefir grains were separated for future use and the filtered KBS drink was stored at 4–8 °C temperature for subsequent analysis.

2.4 Gas chromatography-mass spectrometry (GC-MS) analysis

For the GC-MS analysis, 0.1 g of the KBS sample was mixed with 99% methanol at 27 °C and subsequently subjected to sonication for 2 h. The extracted sample were then filtered using a syringe filter. Agilent-8860 gas chromatography system (Agilent Technologies, USA), coupled with a quadrupole mass spectrometer and employing a DB-5 capillary column was utilised. The carrier gas used was helium at a flow rate of 1 mL/min, and the retention indices were verified using C8–C20 n-alkane series^{28, 29}. The bioactive compounds within the KBS sample were determined by spectrum comparison of the unknown component with known spectra from the National Institute of Standards and Technology library³⁰.

2.5 Rheological analysis

The rheological properties of the samples were assessed using a Brookfield viscometer (DVELV Model, Ametek Inc, MA, USA) equipped with LV-3 spindle. First, 500 mL of the sample was poured into a 600 mL low form Griffin beaker. Measurements were conducted five times at speeds of 6, 12, and 30 rpm at 5 °C and values for both torque (%) and viscosity (mPa.s) were recorded. The shear rate (s⁻¹) was determined through multiplication of the shear rate constant (0.93) with each rpm speed. The shear stress (dyn/cm²) was calculated through multiplication of the torque constant (k=0.09375) with the torque (%). The values of shear stress (dyn/cm²), torque (%) and viscosity (mPa.s) were determined at constant 30 rpm for shelf stability on days 1, 7 and 14. The flow behaviour index values (n) and consistency coefficients (K) of the samples were obtained through a linear regression plot graph according to the power law model using the following equation³¹:

$$\delta = K(\gamma)^n$$

where δ is the shear stress (Pa), K is the consistency coefficient, γ is the shear rate (s⁻¹), and n is the flow behaviour index.

2.6 Statistical Analysis

Each analysis was performed in triplicates, and the results are expressed as mean $\pm$ standard deviation. Plain cow milk kefir prepared using 3% kefir grains (w/v) and, fermented at 25 °C for 24 h was served as the control. Rheological analysis of the KBS and control samples for each shear rates and storage days were performed using Two-way Repeated Measures Anova with Tukey's Post hoc Multiple comparison test. Rheological parameters of power law model between KBS and control was analysed using Student t.test. All the statistical analysis were carried out using Graph Pad Prism 14 version. The p values less than 0.05 as set as statistical significance.

3. Results and Discussions

3.1 GC-MS analysis of KBS

The KBS sample was subjected to GC-MS analysis to identify the bioactive components available in the sample. Figure 1 shows the chromatograph of the GC-MS analysis, which was performed for 22 min. The data given in table -1 includes the 14 chemical compounds with the peak number, retention time area percentage, name of compound, molecular formula, and nature of the compound. The two most abundant compounds, based on area percentage are 6-Oxa-bicyclo [3.1.0]hexan-3-one (37.87%) and 2-Furanmethol (35.9%).

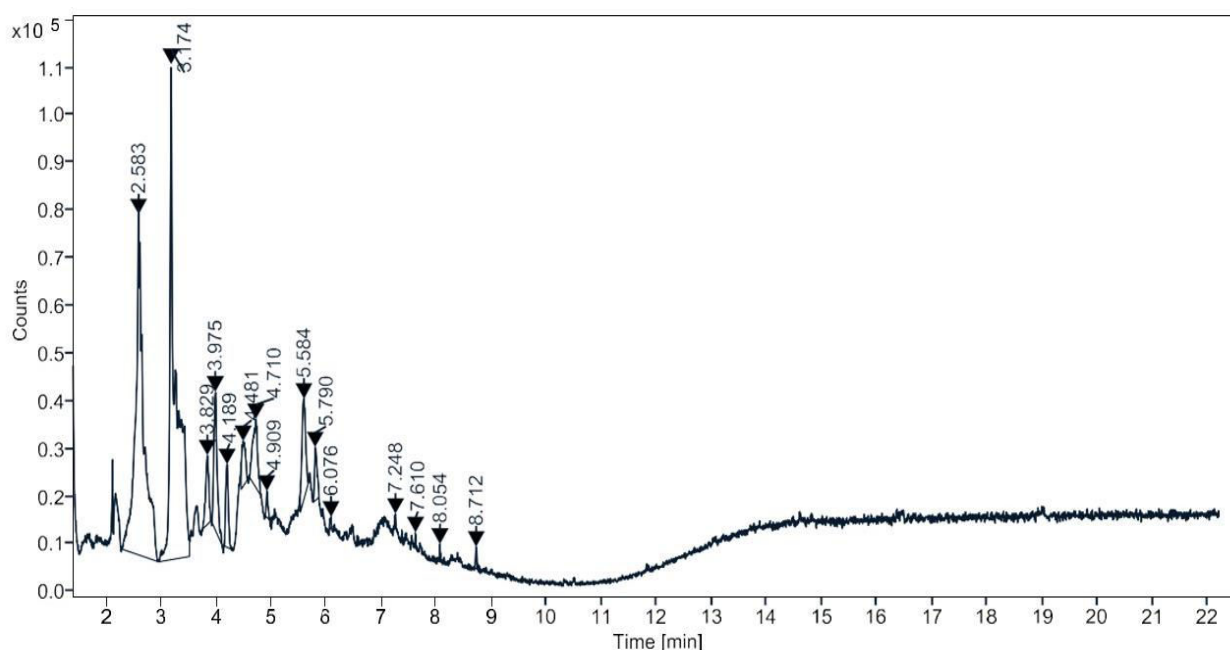


Figure 1: GC-MS chromatogram showing the chemical compound profile of kefir drink supplemented with basil seed mucilage extract. Each peak corresponds to a specific compound, with the x-axis representing retention time (min)—the time taken for each compound to be detected—and indicating area (%), which reflects the relative amount of each compound present in the sample

Table 1: The compositions of bioactive compounds identified in functional kefir drink supplemented with basil seed mucilage.

Peak No.	Retention Time (min)	Area (%)	Compound Name	Molecular Formula	Nature of the compound
1	2.58	35.9	2-Furanmethol	C ₅ H ₆ O ₂	Furan
2	3.17	37.87	6-Oxa-bicyclo[3.1.0]hexan-3-one	C ₅ H ₈ O	Bicyclic lactone
3	4.18	2.92	4H-Pyran-4one, 2-3-dihydro-3,5-dihydroxy-6-methyl	C ₆ H ₈ O ₄	Pyranone
4	3.82	2.81	Butanoic acid, 2-ethyl-2-methyl	C ₇ H ₁₄ O ₂	Free Fatty Acid
5	5.79	1.83	2,4:3,5-Dimethylene-1-iditol	C ₈ H ₁₄ O ₆	Polyol
6	4.48	2.03	D-Fructose, 1,3,6-trideoxy-3,6-epithio	C ₆ H ₁₀ O ₃ S	Fructose
7	5.58	4.95	1-Butene,4-isothiocyanato-1-(methylthio)	C ₆ H ₉ NS ₂	Isothiocyanate
8	4.909	0.61	Ethanol, 2-(2-butoxyethoxy)-acetate	C ₁₀ H ₂₀ O ₄	Ester
9	3.97	5.82	sec-Butyl nitrite	C ₄ H ₉ NO ₂	Nitrite
10	4.71	4.26	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl	C ₁₂ H ₂₂ O ₁₁	Glucose-galactose disaccharide
11	6.07	0.16	Methyl 2,3-di-O-acetyl-4-O-methyl-α-D-xylopyranoside	C ₁₀ H ₁₈ O ₆	Xylose derivative
12	7.24	0.2	2-Acetylamino-3-hydroxy-propionic acid	C ₅ H ₉ NO ₄	Amino acid derivative
13	8.71	0.27	Butyl Citrate	C ₁₈ H ₃₂ O ₇	Citrate ester
14	7.61	0.2	Methy-4,6-di-O-methylmannoside	C ₉ H ₁₈ O ₆	Mannose derivative

The sample contained a diverse mixture of compounds, including bicyclic lactones, furans, disaccharides, esters, amino acid derivatives, mannose and xylose derivatives, pyranones, fatty acids, isothiocyanates, polyols, nitrites, and fructose derivatives. Collectively, these compounds contribute to the bioactive potential of KBS. 6-Oxa-bicyclo [3.1.0] hexan-3-one compound identified is a bicyclic lactone that exhibits anti-

convulsant, anti-microbial, antidiabetic, and anti-obesity effects (This line should go above) ³². 2-Furanmethanol, β -D-glucopyranose, and 4-O- β -D-galactopyranosyl have been reported to possess anti-viral and antioxidant properties ^{33, 34}. The compounds identified were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, butanoic acid, 2-ethyl-2-methyl, and butyl citrate which contribute to anti-inflammatory and antioxidant activities ³⁵⁻³⁷. 2,4:3,5-dimethylene-1-iditol and methyl 2,3-di-O-acetyl-4-O-methyl- α -D-xylopyranoside compounds have been studied for their antimicrobial, anti-mutagenic and anti-cytotoxic potential ^{38, 39}.

Further analysis revealed the presence of 2-acetyl-amino-3-hydroxy-propionic acid (3.44%), a derivative of amino acids with a multifaceted bioactivity profile, including α -glucosidase inhibitory, antioxidant, antimicrobial, and antidiabetic effects ⁴⁰. Finally, the analysis identified methyl-4,6-di-O-methylmannoside (2.94%), a mannose derivative with trans glycosylation activity ³⁹, followed by ethanol, 2-(2-butoxyethoxy)-acetate, 1-butene, 4-isothiocyanato-1-(methylthio), and 2-Acetyl-amino-3-hydroxy-propionic acid with anti-inflammatory, antioxidant, and antiproliferative properties ^{37, 40, 41}.

The previously reported study on cow milk kefir drink have identified furan, ester, lactone, pyranone, polyol, and nitrite groups ^{11, 42} which are consistent with the compounds identified in this study. In contrast, additional studies have identified other bioactive compounds such as polyalkenes, steroids, alkaloids, and aromatic aldehydes, which were not detected in our study ^{42, 43}. This discrepancy could be attributed to various factors, including the inclusion of BSME, kefir grains variety, and production methods.

3.2 Rheological properties

The rheological properties of food products, particularly fermented dairy beverages such as kefir, play a crucial role in consumer acceptability and overall sensory experience ⁴⁴. Therefore, the accurate characterization of these properties is essential for quality control and product development ^{44, 45}. Table -2 presents the rheological measurements of KBS and control samples at different RPM values (6, 12, and 30) having specific shear rate (s^{-1}). As the RPM increased, both the KBS and control samples showed corresponding increase the shear stress (dyn/cm^2).

Rheological parameters such as torque (%), shear stress (Pa), and apparent viscosity ($mPa \cdot s$) were found to be significantly higher in KBS compared to the control at each shear rate ($p < 0.01$). This indicates that KBS possesses higher resistance to deformation and flow under applied shear compared to Control, underscoring its superior rheological performance. Increasing the shear rate resulted in significant increases in rheological parameters within each group, reflecting strong shear-dependent changes (Table 2). KBS maintains a higher viscosity, whereas the viscosity of control samples is lower and shear-dependent (Figure 2). The apparent viscosity of drink decreases as the shear rate increases, which is often desirable for beverages because it contributes to a smoother mouth feel during consumption. The observed range of apparent viscosity values in both KBS and control samples ($1985 \pm 41 mPa \cdot s$ to $296 \pm 7.51 mPa \cdot s$) is consistent

with other studies on fermented kefir drinks^{44, 46} and can be attributed to the weakening of intermolecular forces and structural breakdown within the kefir matrix. However, in few previous studies, there was variation in apparent viscosity values, likely due to the different types of milk used, the specific kefir cultures employed, and the fermentation conditions applied during production^{7, 47}.

Table 2: Rheological measurements of KBS and control with different shear rate(s^{-1})

Parameters	Shear rate (s^{-1})	KBS	Control	p-Value*
Torque (%)	5.58	38.1 $\pm$ 1.21 ^{aA}	14.5 $\pm$ 0.83 ^{aB}	0.01
	11.16	40.3 $\pm$ 1.66 ^{bA}	24.7 $\pm$ 1.90 ^{bB}	
	27.9	44.7 $\pm$ 2.43 ^{cA}	25.6 $\pm$ 1.55 ^{aB}	
Shear stress (Pa)	5.58	3.57 $\pm$ 0.11 ^{aA}	1.35 $\pm$ 0.07 ^{aB}	0.01
	11.16	3.78 $\pm$ 0.15 ^{bA}	2.32 $\pm$ 0.17 ^{bB}	
	27.9	4.15 $\pm$ 0.22 ^{cA}	2.51 $\pm$ 0.14 ^{aB}	
Apparent viscosity (mPa.s)	5.58	1985 $\pm$ 41 ^{aA}	824 $\pm$ 23 ^{aB}	<0.0001
	11.16	557.5 $\pm$ 12.5 ^{bA}	617.5 $\pm$ 18.2 ^{bB}	
	27.9	441 $\pm$ 7.85 ^{cA}	296 $\pm$ 7.51 ^{cB}	

*Two-way ANOVA (Shear rate $\times$ Samples) followed by Tukey's post-hoc multiple comparison test was applied. $p < 0.05$ was considered statistically significant. Means with same letter are not statistically significant, different letters indicate significant difference ($p < 0.05$).

Superscripts in lowercase (a, b, c) show significance across shear rates within each group are significantly different ($p < 0.05$).

Superscript in uppercase (A, B) show significance between groups (KBS vs Control).

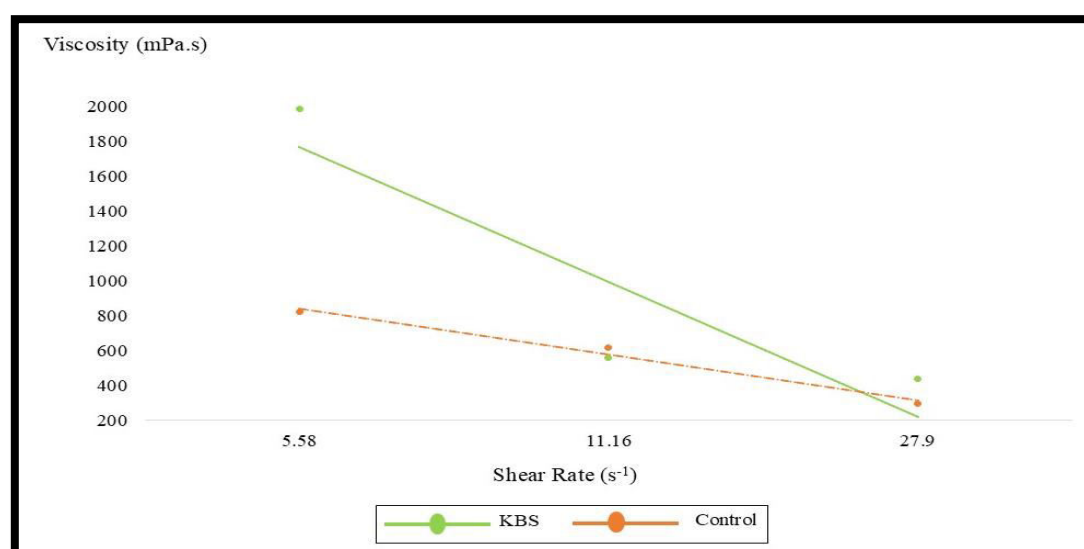


Figure 2: Apparent viscosity of KBS and Control; Kefir drink supplemented with basil seed mucilage extract (KBS); Control (cow milk kefir)

The power law model provided a good fit for the experimental data, as indicated by the high coefficients of determination (R^2) values. This model is frequently used to describe the flow behavior of non-Newtonian fluids, including fermented milk products^{48, 49}. The flow behavior index (n) is a key parameter derived using the power law model, providing insights into whether the fluid is Newtonian ($n=1$), shear-thinning ($n<1$), or shear-thickening ($n>1$)⁵⁰⁻⁵². In this study, both KBS and control samples exhibited shear-thinning behavior ($n=0.032\pm0.001$ and $n=0.661\pm0.01$ respectively), which is typical for fermented dairy products⁴⁴. The flow behavior index for control was significantly higher in comparison to KBS ($p<0.0001$), which indicates KBS is more pseudoplastic (shear-thinning) in nature. Correspondingly, the consistency coefficient (K) was substantially higher in KBS (3.294 ± 0.11) in comparison to control (0.968 ± 0.006) ($p<0.001$), indicating greater viscosity at low shear rates reflective of enhanced flow resistance. The coefficient of determination (R^2) also showed significant improvement for KBS, evidencing a superior fit of the rheological model (Table 3).

Table 3: Estimated rheological parameters of power law model describing flow behaviour index and consistency coefficient

Variables	KBS	Control	95% CI	p-value*
Flow behaviour index (n)	0.032 ± 0.001	0.661 ± 0.01	0.6140 to 0.6506	<0.0001
Consistency Coefficient (K)	3.294 ± 0.11	0.968 ± 0.006	-2.500 to -2.121	<0.0001
Coefficient of determination (R^2)	0.984 ± 0.006	0.92 ± 0.01	-0.08346 to -0.04521	0.0003

KBS: kefir drink supplemented with basil seed mucilage; Control: Cow milk kefir

*Student t-test analysis, $p<0.05$ was considered significant.

The data presented in Table 4 show the impact of storage time on rheological properties for both KBS and control samples. During the interval from day 1 to day 7, no statistically significant changes were observed in torque, shear stress, and apparent viscosity for either group (KBS and control) ($p>0.05$), indicating rheological stability. Followed by day 7 to day 14, there was statistical difference in torque ($p=0.03$), shear stress ($p=0.01$) and apparent viscosity ($p<0.001$) for KBS sample. Previous studies have yielded similar findings, indicating that the viscosity of kefir remains largely stable during short-term refrigeration (up to 7 days), with a noticeable decrease in viscosity occurring after 14 days⁵³⁻⁵⁵. These changes are attributed to progressive protein aggregation and the partial breakdown of the gel matrix, resulting from ongoing post-acidification and microbial interactions⁵⁵.

The torque and shear stress values for KBS were found to be significantly higher than controls at each time point ($p<0.05$) (Table 4). In terms of apparent viscosity, although KBS maintained higher mean values, inter-group differences with control were not

significant ($p=0.0614$) (Table 4). Studies indicates that incorporation of BSM leads to higher gel strength, reduced syneresis, and generally superior rheological stability^{18, 20, 56}. These findings reinforce the observed superior rheological properties of KBS in comparison to control, despite a decline in overall values for both KBS and control during prolonged storage.

Table 4: Rheological measurements of KBS and control with different storage days (1, 7, 14).

Parameters	Storage (Days)	KBS	Control	p-value*
Torque (%)	1	39.7±1.69 ^{aA}	25.6±1.55 ^{aB}	0.0005
	7	42.1±4.9 ^{aA}	28.8±3.14 ^{aB}	
	14	59.5±2.41 ^{bA}	22.4±1.04 ^{bB}	
Shear stress (Pa)	1	3.72±0.15 ^{aA}	2.51±0.14 ^{aB}	0.0005
	7	3.57±0.45 ^{aA}	2.88±0.29 ^{aB}	
	14	5.57±0.22 ^{bA}	2.1±0.09 ^{aB}	
Apparent viscosity (mPa.s)	1	441±7.85 ^{aA}	296±7.51 ^{aB}	0.0614
	7	395.7±3.9 ^{aA}	279±9.4 ^{aB}	
	14	330.5±9.55 ^{bA}	207±8.3 ^{bB}	

KBS: kefir drink supplemented with basil seed mucilage; **Control:** Cow milk kefir

*Two-way ANOVA (Days × Samples) followed by Tukey's post-hoc multiple comparison test. $p<0.05$ was considered significant. Means with same letter are not statistically different, different letters indicate significant difference ($p<0.05$).

Superscripts in lowercase (a, b, c) show significance across time (Days 1, 7, 14 within each group) are significantly different ($p<0.05$).

Superscript in uppercase (A, B) show statistical significance between groups (KBS vs Control).

4. Conclusions

This study provides a comprehensive characterization of a synbiotic beverage combining kefir with BSME. The GC-MS analysis elucidated the volatile compound profile, revealing contributions from kefir of organic acids, esters, ethanol and unique polysaccharide components from the inclusion of BSME. The integration of BSME significantly altered the rheological properties of kefir, enhancing its viscosity and consistency, as evidenced by the power law model parameters. The KBS exhibited shear-thinning behavior compared to the control, which was attributed to the complex polysaccharide network of the BSM. This enhancement is crucial for improving mouth feel and consumer acceptability. The insights gained from this study have implications in the development of novel synbiotic beverages with improved functional and sensory properties. Additionally, the storage stability revealed degradation starting at day 14 of

rheological properties. However, the influence of varying storage conditions, in-vitro assessments of antioxidant and antimicrobial activities have not been investigated in the study, thereby limiting comprehensive insights. Future research should focus on investigating the stability of KBS during storage and their impact on the viability of probiotic bacteria.

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6. Conflict of Interest

There is no conflict of interest.

7. References

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