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Formulation of Two Herbal Probiotic Water Kefir Beverages and Evaluation of Their Physicochemical Parameters and Sensory Characteristics

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Abstract
This study investigates the formulation, physicochemical properties, and sensory evaluation of two herbal water kefir beverages, infused with basil leaves and amla. Water kefir, a nondairy probiotic beverage, is gaining popularity for its health benefits and suitability for consumers avoiding dairy. The addition of herbs such as basil, amla, ginger, mint, and lemon powder aim to enhance the beverage's nutritional and functional properties. Both formulations were assessed for pH, Total Soluble Solids (TSS), acidity, alcohol content, and key nutritional components, including vitamins. Sensory evaluation was conducted by a trained panel, focusing on taste, aroma, color, texture, and overall acceptability. Results revealed that basil water kefir had a higher preference due to its mild sweet flavor and smooth texture, while amla kefir demonstrated a tangy and sour taste and moderate vitamin C content. Correlation analysis indicated that physicochemical properties like acidity and TSS significantly influenced sensory attributes, impacting consumer acceptance. The study concludes with recommendations for the production of herbal water kefir, highlighting its commercialization potential as a functional beverage and suggesting further research on additional herbal infusions to expand market appeal.

Keywords: Water kefir, probiotic beverage, herbal, amla Water kefir; basil Water kefir; Physicochemical properties; Sensory evaluation; Consumer acceptability; Functional beverage.

1. Introduction

a) Background on Water Kefir

Water kefir is a naturally fermented, non-dairy probiotic beverage, produced through the metabolic activities of beneficial bacteria and yeasts in a symbiotic culture known as "kefir grains." Unlike dairy-based kefir, water kefir is prepared using a sugar-water solution, which is fermented over a period of 24-48 hours. This fermentation process results in a mildly acidic and carbonated drink that offers several health benefits due to its rich probiotic content, which contributes to gut health, immune system support, and overall wellness (Bourrie *et al.*, 2016). With the increasing focus on functional foods and beverages, water kefir has gained popularity as a health-promoting alternative to sugary soft drinks and as an option for those who prefer or require a dairy-free diet (Gómez-Constanzo *et al.*, 2021).

b) Significance of Herbal Probiotics

Incorporating herbs into probiotic beverages like water kefir provides added benefits beyond standard probiotics, as herbs contain bioactive compounds that can enhance the beverage's therapeutic properties. Herbs such as ginger, mint, and basil are known for their antioxidant, anti-inflammatory, and antimicrobial properties, which may complement the probiotic effects of kefir, potentially providing a synergistic effect on health outcomes (de Almeida *et al*., 2019). Additionally, herbs can enhance the sensory properties of water kefir, adding unique flavors and aromas that improve its palatability and consumer appeal, making it more acceptable to a broader audience (Silva *et al*., 2022).

c) Objectives of the Study

The primary objectives of this study are to:

1. Formulate two herbal water kefir variants by integrating selected herbs into the kefir fermentation process.
2. Evaluate the physicochemical properties of each formulation, including pH, total soluble solids, acidity, and nutritional content.
3. Conduct a sensory evaluation to assess consumer acceptability based on taste, aroma, color, texture, and overall preference.

2. Literature Review

a) **Health Benefits of Probiotics**

Probiotics are live microorganisms that provide numerous health benefits when consumed in adequate amounts, particularly by supporting gut health, enhancing immune function, and potentially preventing various diseases (Hill *et al*., 2014). The consumption of probiotic-rich foods is associated with a balanced gut microbiota, which plays a crucial role in digestive health and helps prevent gastrointestinal disorders such as irritable bowel syndrome and diarrhea (Sanders *et al*., 2018). Moreover, probiotics have been found to modulate the immune system, reduce inflammation, and may even improve mental health, linking gut health to broader systemic benefits (Kleerebezem *et al*., 2019).

b) **Water Kefir as a Functional Beverage**

Water kefir is a non-dairy probiotic beverage that contains a rich diversity of beneficial bacteria and yeasts, including Lactobacillus, Leuconostoc, Acetobacter, and Saccharomyces species (Gómez-Constanzo *et al*., 2021). The fermentation process in water kefir involves converting sugars into organic acids, gases, and other compounds that contribute to its characteristic flavor, mild acidity, and slight carbonation. Research on water kefir has highlighted its potential as a functional beverage due to its probiotic content, low calorie and sugar levels, and high antioxidant activity (Corona *et al*., 2016). The presence of beneficial microbes in water kefir supports its use as an alternative probiotic source, especially for those with dairy intolerances (Gulitz *et al*., 2011).

c) **Role of Herbs in Probiotic Beverages**

Herbs are often added to probiotic beverages to enhance their nutritional and therapeutic properties. Herbs such as ginger, mint, and basil offer unique flavors and contain bioactive compounds that may work synergistically with probiotics to promote health (de Almeida et al., 2019). For example, ginger has anti-inflammatory and digestive health benefits, while mint is known for its soothing properties and potential to aid digestion (Rafieian-Kopaei *et al*., 2018). The addition of herbs to probiotic drinks like water kefir can boost antioxidant capacity, provide additional vitamins and minerals, and improve microbial viability, thereby enhancing the overall efficacy and health benefits of the beverage (Silva *et al*., 2022).

Basil Leaves (Ocimum basilicum): Basil is an aromatic herb extensively used in traditional medicine for its health benefits. It is used to treat respiratory disorders and digestive issues and as a general health tonic. Basil leaves are rich in essential oils, including eugenol, linalool, and methyl chavicol, along with flavonoids and phenolic acids. Scientific research has substantiated the anti-inflammatory, antimicrobial, and antioxidant properties of basil leaves, showing their efficacy in supporting cardiovascular health, improving digestion, and boosting the immune system (Lee and Umano, 2005).

Amla (Phyllanthus emblica): Amla, or Indian gooseberry, is a cornerstone of Ayurvedic medicine, known for its potent antioxidant properties, immune-boosting effects, and benefits for digestion and skin health. It is exceptionally rich in vitamin C, polyphenols, tannins, and flavonoids. Extensive research supports Amla's health benefits, including its ability to lower cholesterol, enhance liver function, and bolster immunity. Its high antioxidant content helps reduce oxidative stress and inflammation, making it a valuable addition to health regimens (Baliga and Dsouza, 2011).

3. Materials and Methods

1. Formulation of Amla and Basil water kefir

a) Ingredients and Selection of Herbs

1. **Kefir Grains**: Live cultures of bacteria and yeast, the primary source of fermentation.
2. **Base Solution**: A sugar-water solution (10% w/v), serving as the substrate for microbial fermentation.
3. **Herbs Selection**: Two herbs were chosen based on their health benefits and flavor compatibility with kefir.
    1. **Amla** *(Phyllanthus emblica),* known for its potent antioxidant properties, immune-boosting effects, and benefits for digestion and skin health.
    2. **Basil leaves** *(Ocimum sanctum) are* used to treat respiratory disorders and digestive issues and as a general health tonic.
    3. **Ginger (Zingiber officinale),** known for its anti-inflammatory and digestive properties.
    4. **Mint (*Mentha piperita*)** for its refreshing aroma and potential digestive benefits.

b) Preparation Process

1. **Preparation of Sugar Solution**: Dissolve sugar in distilled water to create a 10% w/v solution.
2. **Formation of kefir base**: Add kefir grains (5% w/v) to the sugar solution. Ferment for 24 hours and store at refrigerator temperature.
3. **Herbal Sachet formation**: Add 5 grams of selected herbs to each variant, ensuring thorough mixing. 2.5 gm apple powder, 0.5 gm mint powder, 0.5 gm ginger powder, 0.5 gm lemon powder. The ratios of these powders will be carefully balanced to create the desired flavor. Appropriate quantity of all powders was then used in different ratios in the preparation of the herbal sachet.
4. **Formation of herbal beverage**: Pour the solution into sterilized glass jars ,grains were taken out ,dip the herbal powder sachet in the base kefir and cover with a breathable cloth to allow for gas release while preventing contamination.

c) Fermentation Process

- **Duration**: The jars were fermented at room temperature (25-30°C) for 24-48 hours.
- **Temperature**: Monitored to maintain a consistent environment for optimal microbial activity.
- **Kefir Grain Preparation**: The grains were rinsed with distilled water before use and stored in fresh sugar solution between fermentations.

a) **Physicochemical and Nutritional Analysis**

1. **pH Measurement**: Using a calibrated digital pH meter, measure the pH of each sample at the end of fermentation.
2. **Total Soluble Solids (TSS)**: Use a refractometer to determine the TSS, reported in degrees Brix, indicating the sugar concentration in the sample.
3. **Acidity**: Titration with 0.1 N NaOH to determine the percentage of lactic acid, indicating the level of acidity.
4. **Alcohol Content**: Use gas chromatography or an alcohol meter to measure the ethanol concentration formed during fermentation.
5. **Sugars**: High-performance liquid chromatography (HPLC) was used to analyze residual sugar levels post-fermentation.
6. **Vitamins**: Vitamin C and B-group vitamins were measured using HPLC to evaluate nutritional enhancements from the herbs.
7. **Mineral Content**: Atomic absorption spectroscopy (AAS) was employed to quantify essential minerals such as calcium, magnesium, and potassium.

b) **Sensory Evaluation**

Sensory evaluation is critical in determining the acceptance and preference of new food products by consumers. Key sensory attributes, such as taste, aroma, color, texture, and overall appearance, significantly influence consumer choices (Lawless & Heymann, 2010). In the context of probiotic beverages, pleasant sensory attributes can enhance palatability and encourage regular consumption, thereby supporting the health benefits of probiotics (Breslin et al., 2013). Sensory evaluation methods, such as the use of trained panels or consumer acceptance testing, are widely used in food studies to assess and optimize sensory characteristics, making sensory evaluation an essential step in product development (Stone *et al*., 2012).

A sensory panel was used to assess the organoleptic qualities of each water kefir variant.

Samples were presented in a randomized order to reduce bias.

Panel Selection and Training

- **Panellists**: A group of 10 trained individuals aged 20-45 was selected based on familiarity with sensory evaluation methods.
- **Training**: Panellists were trained in a brief session on identifying key sensory attributes specific to probiotic beverages, including basic flavor, aroma, and mouthfeel descriptors.

Scoring Parameters

- **Taste**: Evaluated for sweetness, acidity, and any distinct herbal flavors.
- **Smell**: Assessed for pleasantness and herbal distinctiveness.
- **Appearance**: Measured visually and scored for clarity, vibrancy, and appeal.
- **Texture**: Scored for mouthfeel, including any effervescence and thickness.
- **Overall Acceptability**: A comprehensive score representing the panelists' overall satisfaction with each variant.

3.Explanation of Parameters

Parameter	Amla Water Kefir	Basil Water Kefir	Explanation
pH	3.97	3.77	Indicates acidity level; a lower pH suggests higher acidity.
Total Soluble Solids (°Brix)	1.47	3.83	Measures sugar concentration; lower values suggest more fermentation.
Acidity (%)	3.92	4.8	Higher acidity reflects more fermentation by kefir grains.
Alcohol Content (%)	2.9	3.12	Indicates mild alcoholic content from fermentation.
Sugar Content (g/L)	1.9	3.55	Measures residual sugar after fermentation.
Vitamin C (µmg/L)	93.77	98.2	Shows vitamin C contribution from the herbs.
Vitamin A(µmg/L)	7.94	17.5	Measures vitamin A, reflecting contributions from both kefir and herbs.

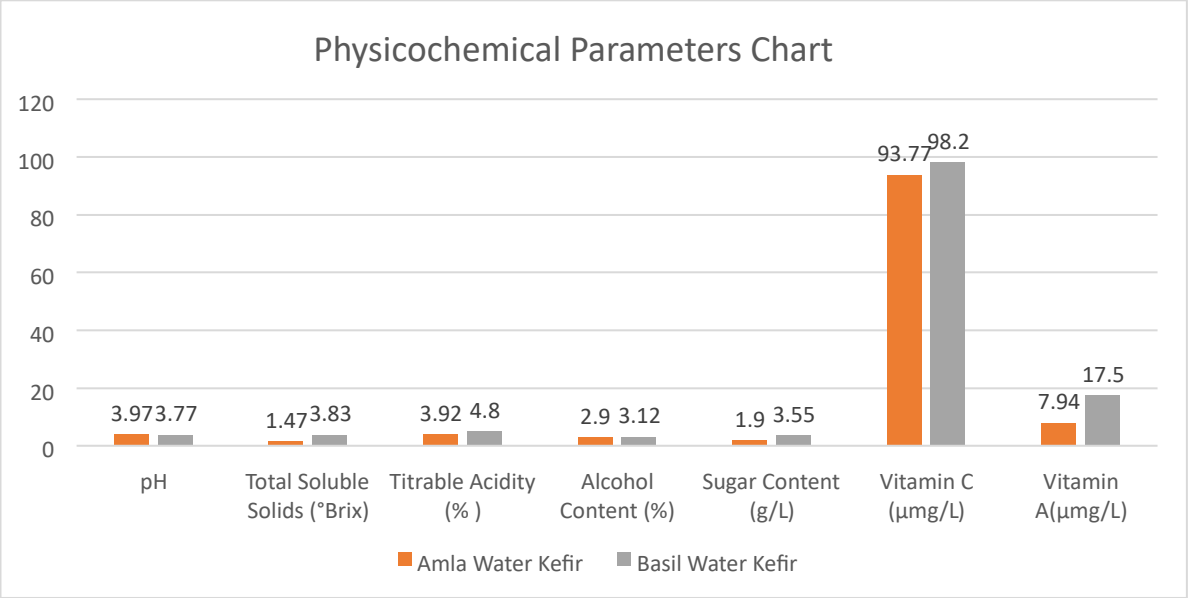
Sensory Attribute	Amla Water Kefir	Basil Water Kefir	Explanation
Taste (9-point scale)	7.1	8.6	Evaluates the flavour balance between acidity and herbal taste.
Smell (9-point scale)	7.4	8.6	Reflects the pleasantness and strength of herbal aroma.
Appearance (9-point scale)	7.2	8.7	Assesses the visual appeal and clarity of the beverage.
Texture (9-point scale)	7.4	8.7	Measures mouthfeel, including carbonation and smoothness.
Overall Acceptability (9-	7.275	8.65	Overall score indicating consumer

point scale) preference.

a) Physicochemical and Nutritional Parameters

1. **pH**: Reflects the acidity of the beverage. Basil kefir has a slightly lower pH (3.77), indicating a more acidic profile than Amla kefir (3.97).
2. **Total Soluble Solids (°Brix)**: Indicates residual sugar levels. Basil water kefir shows the highest Brix levels, with Amla kefir being lower, suggesting a slightly sweeter profile for basil water kefir.
3. **Acidity (% (lactic acid)**: Represents the lactic acid produced by fermentation, contributing to the tangy taste of kefir. Basil kefir has a marginally higher acidity.
4. **Alcohol Content**: A small amount of alcohol is produced during fermentation. Both variants have minimal alcohol, making them suitable for non-alcoholic categorization.
5. **Sugar Content (g/L)**: Measures residual sugars left after fermentation. Amla kefir has slightly lower sugar content than basil kefir, suggesting slightly more complete fermentation.
6. **Vitamin C (µg/L)**: Herbs contribute additional nutrients. Basil kefir has a higher vitamin C level.
7. **Vitamin A (µg/L)**: Basil kefir has a higher vitamin A level.

Physicochemical Parameters Chart: This chart shows values for pH, Total Soluble Solids (°Brix), Acidity, Alcohol Content, Sugar Content, Vitamin C, and Vitamin A Content, allowing for a direct comparison between the Amla and Basil kefir.



This figure illustrates a comparative analysis of physicochemical attributes between basilinfused and amla-infused water kefir formulations. Parameters include pH, total soluble solids (TSS), titratable acidity, alcohol content, sugar concentration, and vitamin levels (A and C).

Table 1: Physicochemical Parameters of Amla Water Kefir and Basil Water Kefir

Group	TSS (%)	Titratable Acidity (%)	Alcohol (%)	CO_2 (%)
Amla kefir	1.47 ± 0.01^{c}	3.92 ± 0.03^{b}	2.90 ± 0.08^{c}	1.27 ± 0.04^{c}

Basil kefir	3.83 ± 0.02[a]	4.80 ± 0.03[a]	3.12 ± 0.09[b]	2.69 ± 0.06[a]

Table 2: Vitamin Analysis of Amla Water Kefir and Basil Water Kefir

Group	Vit A(µmg/L)	Vit C(µmg/L)
Amla kefir	7.94 ± 0.3[b]	93.77 ± 1.5[b]
Basil kefir	17.5 ± 0.5[a]	98.2 ± 1.5[a]

Physicochemical parameters and vitamin analysis have been shown in Table 1 and Table 2, respectively. As shown in Table 1, basil water kefir exhibited higher TSS and acidity compared to its amla counterpart, indicating more robust fermentation

Flavor and Sweetness: Basil's higher TSS suggests greater residual sugars or soluble nutrients, possibly contributing to better taste and mouthfeel—consistent with sensory scores.

Acidity & Preservation: Elevated titratable acidity improves shelf life and delivers a zesty profile—valuable for formulation.

Fermentation Yield: More alcohol and CO_2 in Basil kefir mean its microbial communities (likely yeasts and LAB) are more active or better adapted—worth exploring via microbial profiling.

Vitamin A: Basil kefir's vitamin A is **~120% higher** than Amla kefir—crucial for vision, skin health, and immune regulation.

Vitamin C: Both samples are excellent sources, but basil still outpaces amla—suggesting enhanced antioxidant potential and shelf-life stability.

b) Sensory Parameters

Table 3: Sensory Parameters of Basil Kefir and Amla Kefir

Sample	Appearance	Taste	Smell	Texture	Overall Acceptability
Basil Kefir	8.7 ± 0.1[a]	8.6 ± 0.2[a]	8.6 ± 0.2[a]	8.7 ± 0.2[a]	8.65 ± 0.1[a]
Amla Kefir	7.2 ± 0.2[b]	7.1 ± 0.1[b]	7.4 ± 0.2[b]	7.4 ± 0.2[b]	7.275 ± 0.1[b]

Significant at $P < 0.05$; values followed by different superscript letters within the same row denote a significant difference.

Table 3 shows a sensory comparison between Basil Water Kefir and Amla Water Kefir.

Basil Kefir scores: consistently high across attributes (8.6–8.7). It leads across every sensory metric, suggesting stronger consumer acceptability. The elevated scores in texture and aroma may be due to basil's aromatic compounds and smoother fermentation profile. Basil's higher °Brix and acidity likely contribute to enhanced flavor and aroma perception.

Amla Kefir scores: moderately high (7.1–7.4), but noticeably lower than Basil.

Sensory Evaluation Chart: This chart displays the sensory scores for taste, aroma, color, texture, and overall acceptability, highlighting consumer preferences and sensory attributes for each variant. Sensory analysis revealed that **basil water kefir** scored significantly higher across all attributes when compared to **amla kefir** ($P < 0.05$). Specifically, basil kefir excelled in appearance (8.7), taste (8.6), and texture

(8.7), reflecting superior visual appeal and mouthfeel. Its elevated overall acceptability score (8.65) underscores broad consumer preference.
It was consistent across panellists, with basil kefir often receiving the superscript **"a"**, confirming its statistically significant preference in pairwise comparisons.

4. Statistical Analysis

The sensory analysis demonstrated the statistically significant differences between appearance, taste, smell, texture, and overall acceptability (p 0.05), which clearly shows the influence of herbal enrichment on the organoleptic characteristics of kefir beverages.
Whenever one-way ANOVA indicated a statistically significant F-value ($p \leq 0.05$), post hoc comparisons were performed using Tukey's Honest Significant Difference (HSD) and Duncan's Multiple Range Test (DMRT). These tests facilitated pairwise evaluations and treatment grouping, with superscripted alphabetical markers (e.g., ^{a}, ^{b}, ^{c}, ^{d}) denoting significant differences. Basil water kefir consistently received the superscript '^{a}' across sensory attributes, signifying its statistically superior acceptability over other formulations.

5. Results

The physicochemical properties of the amla and basil water kefir variants were analyzed to assess their quality and functional attributes. Both variants exhibited slightly acidic pH levels, with the Amla kefir recording a pH of 3.97 and the Basil kefir at 3.77. These levels indicate suitable acidity for probiotic beverages, as an acidic environment supports the growth of beneficial microorganisms and prolongs shelf life (Corona *et al*., 2016). The Total Soluble Solids (TSS) values, representing residual sugars, were 1.47 °Brix for Amla water kefir and 3.83 °Brix for Basil water kefir, suggesting a mild sweetness that remained after fermentation.
Titrable acidity was slightly higher in basil water kefir (4.8%) than in amla water kefir (3.92%), indicating more robust fermentation in the former. Alcohol content was slightly higher in both variants, with Basil Water kefir containing 3.12% and Amla Water kefir 2.9%, possibly due to secondary fermentation.
In terms of nutritional composition, Basil Water kefir demonstrated a higher vitamin C content (98.2 µg/L) compared to Amla Water kefir (93.77 µg/L). Vitamin analysis revealed that Basil Water kefir had a slightly higher concentration of Vitamin A (17.5 µg/L) compared to Amla Water kefir (7.94 µg/L).
The sensory evaluation assessed taste, smell, appearance, texture, and overall acceptability of the two water kefir variants. Panelists rated the basil water kefir variant slightly higher in taste, with a score of 8.6, as its flavor was perceived as milder and more refreshing, while amla water kefir scored 7.1. Smell scores were close, with basil water kefir scoring 8.6 due to its strong herbal scent, slightly exceeding amla water kefir's 7.4 (Stone et al., 2012). For color, Basil Water kefir was marginally preferred (8.7) for its clarity and subtle red-brownish tint, as compared to Amla Water kefir (7.2).
Texture scores favored basil Water kefir at 8.7, reflecting its smooth mouthfeel, while Amla Water kefir scored 7.4. Overall acceptability scores further indicated a consumer preference for Basil Water kefir, scoring 8.65 against Amla Water kefir's 7.275.
Statistical analysis revealed significant differences in the sensory attributes of taste, texture, and overall acceptability between the two variants ($p < 0.05$), suggesting that the panelists showed a slight preference for Basil Water kefir due to its milder sweet flavor profile and refreshing smell of basil leaves.

Additionally, the pH and TSS levels influenced texture and overall acceptability, with Basil Water kefir's slightly higher TSS aligning with a smoother mouthfeel and greater overall satisfaction among the participants (Lawless and Heymann, 2010). This suggests that while both formulations were well-received, variations in physicochemical properties had a tangible effect on sensory perception and consumer preference.

6. Discussion

The physicochemical properties of both amla and basil water kefir variants demonstrated slight deviations from standard water kefir values, which can be attributed to the addition of herbs. Though both are potent, basil's higher vitamin A and C levels suggest it could be marketed as a stronger antioxidant beverage. Additionally, the pH and TSS levels influenced texture and overall acceptability, with basil water kefir's slightly higher TSS aligning with a smoother mouthfeel and greater overall satisfaction among the participants (Lawless and Heymann, 2010). This suggests that while both formulations were well-received, variations in physicochemical properties had a tangible effect on sensory perception and consumer preference.

From a health perspective, the acidity and pH levels of both beverages are beneficial, as they create an environment favorable for probiotic survival, potentially enhancing gut health (Sanders *et al*., 2018). Ginger content may also provide additional antioxidant benefits, further supporting immunity and potentially reducing inflammation (Rafieian-Kopaei *et al*., 2018).

The addition of ginger and mint distinctly influenced the sensory attributes of each variant. Ginger's pungency contributed to a stronger, spicy aroma and a slightly more intense taste, which some panelists appreciated for its robustness but others found too potent (Silva *et al*., 2022). In contrast, mint provided a refreshing, mild flavor and aroma, resulting in higher scores for taste and overall acceptability due to its more subtle profile.

7. Conclusion

This study evaluated the physicochemical properties and sensory attributes of two herbal water kefir variants, basil water kefir and amla water kefir, providing insights into their formulation and consumer acceptability. Both variants showed suitable pH levels, slightly high alcohol content, and beneficial acidity, aligning with the standards of non-dairy probiotic beverages. The basil variant displayed higher acidity and vitamin C and vitamin A levels, likely due to basil's active compounds, while amla kefir had a smoother mouthfeel and slightly higher alcohol and sugar content, contributing to its greater aroma.

Sensory evaluation revealed that basil water kefir was preferred overall, particularly for its mild, refreshing flavor and smoother texture, making it suitable for a broader consumer base.

amla water kefir, with its tangy flavor and higher nutrient profile, may appeal to healthconscious consumers seeking potent functional benefits.

For future production, we recommend using basil and amla and other mild herbs for water kefir formulations aimed at new consumers of probiotic beverages. Both variants have commercialization potential in the growing functional beverage industry, meeting consumer demand for non-dairy, probiotic-rich, and nutritionally enhanced drinks. Further research could explore additional herbal variants, optimize fermentation conditions, and conduct largescale consumer studies to refine formulations and expand market appeal.

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