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FORMULATION AND EVALUATION OF HERBAL WATER KEFIR BEVERAGE USING GUAVA LEAF POWDER: A STUDY ON FUNCTIONAL AND SENSORY ATTRIBUTES

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Abstract
The main aim of this study is to evaluate the nutritional composition, physicochemical parameters, and sensory analysis of the new herbal water kefir beverage, guava water kefir beverage. Plain kefir is used as a base formulation, and guava kefir drink is developed by adding selected medicinal herbs with bioactive components. Nutritional analysis of a plain water kefir and herbal combination of different herbal powders, ginger, mint leaves, and lemon powder was carried out using the standard chemical methods. The nutritional and organoleptic properties of guava-based kefir beverages that have been fermented and kept at room temperature and in a cool environment are examined in this study. According to vitamin evaluation, thermolabile substances, including vitamin B12 (3.75 ± 0.19 µg/mL vs. 3.11 ± 0.62 µg/mL) and vitamin C (93.00 ± 0.40 µg/mL vs. 92.00 ± 0.85 µg/mL), were significantly preserved during cold storage. Guava kefir's physicochemical analysis revealed moderate acidity (titratable acidity: 4.36%) and regulated ethanol production (2.82%–3.58%), in contrast to the control samples' greater levels of carbonation and alcohol. While cool storage reduced metabolite overproduction, room temperature settings improved microbial fermentation indicators. Using a 9-point hedonic scale, the sensory evaluation produced consistently high scores for look, taste, smell, and texture, as well as an overall acceptance of 7.675 ± 0.2 with little statistical variation ($p > 0.05$). The formulation and analysis of a guava water kefir herbal beverage, incorporating guava leaves, represents a novel approach in creating a functional beverage with enhanced nutritional and health benefits. This research paper will deal with the formulation of herbal plant powder and utilize it in healthy probiotics as well as an innovative water kefir drink, which will lead to the development of a healthy beverage. It developed a new herbal probiotic beverage from herbal plants, which will provide novel uses for underutilized plants. It will further provide consumers with new alternatives to probiotic herbal beverages. Moreover, this research will bring light to the potential of medicinal plants for new food product development.

Keywords: *Beverage, herbal, guava water kefir, bioactive, antioxidant, beverage*

1 Introduction to Herbal Water Kefir

Water kefir is a traditional fermented beverage that is refreshingly sour and slightly carbonated. It usually has less than 1% alcohol and up to 2% lactic acid. It develops when water kefir grains ferment sugar; dried fruits are often added to the fermentation process (Moretti *et al.*, 2022).

There is no sufficient scientific literature specified for water kefir, its origin, microbial and chemical structure, and the standard method of preparation.

Water kefir may have originated in Mexico, Tibet, the Ginger Beer Plant, Tepache, France, Italy, or Africa. French biologist M. L. Lutz discovered kefir grains in Mexico in 1899, leading to scientific documentation. Despite its specific origins being unknown, the beverage may have gained popularity in France in the early 1900s. The few findings that have been obtained about water kefir focused on the microflora and chemical structure of sugar kefir drink and sugary

Brazilian kefir, brown sugar kefir, sugary kefir grain (ginger beer plant) in France, tobiko (sugary kefir), and water kefir in Germany. Although its historical origin is uncertain, water kefir grains have been passed down through the generations, and they are quite preferred in various countries such as Japan, Thailand, Malaysia, the United Kingdom, Spain, the Netherlands,

Brazil, Chile, Peru, and Argentina (Fiorda *et al.*, 2017). Water kefir is a beverage produced by the fermentation of water and kefir grains. The beverage can be consumed as a natural probiotic drink due to its health-promoting properties. It is an indigenous method of producing lightly alcoholic beverages with diverse origins. All natural sugars typically utilized for kefir water production are mixed with water, and the mixture is then incubated to reach the desired quality. Previous studies have reported that commercial kefir water has reached marketability; this discovery has led to great demand for a stable supply of standardized kefir water. Additionally, as the microorganisms in water kefir grains are extremely adaptable to a variety of sources, it is reported that a wide range of sugar sources can be used to produce water kefir (Bueno *et al.*, 2021). Previous studies have mainly focused on the production and fermentation of kefir water from various types of natural sugars, i.e., sugarcane juice, coconut water, saccharose, and stevia sweetener (Tavares *et al.*, 2023). Water kefir has been associated with various health benefits, including improved gut health, immune system support, and anti-inflammatory properties (Rosa *et al.*, 2017). The potential health benefits of water kefir make its bacterial composition of particular interest. It has been shown that lactic acid bacteria, including Lactobacillus, have probiotic properties and can boost the immune system and promote gut health (Hill *et al.*, 2014).

Due to the commercial success of water kefir, there is now a high demand for stable kefir water production, so the production of herbal water kefir using herbs is gaining increasing interest. Moreover, numerous studies have been published demonstrating the physiological effects of kefir water. Combining the probiotic potential of water kefir with the phytochemical richness of herbs and spices can create a unique and functional beverage. Additionally, kefir grains are known to be very diverse in their biochemical characteristics, which are highly induced by characteristics of the habitat.

1.1 SELECTION OF MEDICINAL PLANTS FOR THE STUDY

Guava Leaves (Psidium guajava L.)

The guava plant is a creamy evergreen shrub that grows in Latin and Central America. The crop is also cultivated in other countries such as Egypt, India, Cuba, the Canary Islands, China, and Hawaii. In Malaysia, guava fruits and leaves are used in traditional medicine and 'rendang' as an ingredient that will not spoil the 'rendang.' Dried guava leaves are used in Malaysia for brewing a healthy, dark-coloured guava leaf tea beverage. The benefits and restrictions of the guava leaf tea beverage are not widely known in Malaysia. Young guava leaves contain a flavonoid antioxidant that is called quercetin. Essential oils are found in the leaves. Polyphenols, including catechin, which has numerous health benefits, including reducing the risk of heart disease and cancer. The health benefits of the polyphenols may also include reducing blood sugar levels. (Kumar *et al.*, 2021) (Patil *et al.,* 2023) (Dwiwina *et al.*, 2023) The nutritional content of guava leaves includes tannins, which naturally add texture and help retain the value and preserve the quality of beverages. Additionally, the extract is high in vitamin C, offering potential anti-inflammatory and antioxidant effects, as well as benefits for animal health. Research suggests that using guava leaf extract to brew mineral water could create a functional beverage with natural ingredients, supporting overall health and ecological balance. (Upadhyay and Dass 2020) (Rani & David, 2021)

The guava (Psidium guajava L.) tree belongs to the family Myrtaceae and is primarily found in the Neotropics. Guava fruit, aside from its nutritional importance, has huge potential with respect to its pharmacological and functional attributes. Guava leaves have been utilized by numerous cultures due to their natural therapeutic effects. Guava leaves and fruit are a valuable source of bioactive compounds such as polyphenolic compounds. Guava leaves have strong anti-ulcer and gastroprotective, analgesic, anti-inflammatory, antidiabetic, antimicrobial, antihypertensive, and antioxidant effects. Guava leaves combined with black and green tea are a functional beverage rich in polyphenolic compounds and supportive for reducing the metabolic changes occurring in animals consuming high-fat diets. (Ibeh *et al.* 2021) (Shady *et al.*, 2022).

Mint Leaves (Mentha spp.)

Mint leaves, from the Mentha genus, are widely appreciated in traditional medicine for their soothing and therapeutic properties. They are commonly used to treat digestive issues, including indigestion, gas, and irritable bowel syndrome (IBS), due to their ability to relax the muscles of the gastrointestinal tract. Rich in essential oils such as menthol, menthone, and limonene, mint leaves exhibit significant antimicrobial, anti-inflammatory, and antioxidant properties. Scientific studies have confirmed that mint leaves can help alleviate symptoms of digestive discomfort, enhance respiratory health, and provide a calming effect, making them a valuable addition to both culinary and medicinal applications (McKay & Blumberg, 2006). Their refreshing aroma and flavour also contribute to their widespread use in food and beverages.

2 Materials and Methods

2.1 Materials

a) Guava leaves and mint leaves were taken from home garden. Apple, ginger and lemon were acquired from the market and brought to the Food and Nutrition Department at the
Bhagat Phool Singh Mahila Vishwavidyalaya University.

b) All leaves were washed and sundried then grinded into fine powder and stored. After being peeled and washed, the ginger, apple, and lemon were dried in the gentle sunlight.
Ginger, Apple and lemon were peeled, washed and dried in sun shaded light.

c) After properly drying process they are grinded in to fine powders. Appropriate quantity of all powders was then used in different ratios in the preparation of the herbal sachet.

d) Water kefir grains were bought online at amazon. (figure 2.1a)

e)

Figure 2.1(a) Water Kefir Grains (Image was captured by Galaxy S21 Ultra 5G)

2.2 Herbal Beverage Preparation

Two batches of beverages were prepared

I Plain water kefir available in the market (figure 2.2a)

II All herbal based water kefir beverage (figure 2.2b)

The First batch was prepared by adding Water Kefir grains and Water along with brown sugar and molasses included in the kit. Ferment for 24 hours and store at refrigerator temperature. In the Second batch, 3.5 grams of kefir grains were added to 400 ml of water along with 7.5 grams of white sugar, 3.75 grams of brown sugar and 3.75 grams of palm sugar. 5 ml of beetroot extract was added to improve the color. Fermented at room temperature (34 degrees Celsius) for 24 hours

Figure 2.2a First Batch of Plain Water Kefir Available in the Market (Image was captured by Galaxy S21 Ultra 5G)

2.3 Formulation of Guava leaves powder

a)4gram of Guava leaves powder ,2.5 gram of Apple Powder .0.5 gram of Mint powder, 0.5 gram of Ginger powder and 0.5 gram of Lemon Powder.

b) All formulated powder were added in cloth bags sachet and stored (figure 2.3b)

c)After 24 hours of fermentation kefir grains are strained out and stored for future use

d)Guava herbal sachet (figure 2.3a) which was prepared is dipped in Jar of water kefir batch for
5 hours and after 5 hours it is taken out.

e) After that both bottles were stored at refrigerated temperature.

Figure 2.3a Sachet of Herbal powder dipped in Water Kefir Beverage (Image was captured by Galaxy S21 Ultra 5G)

2.4 Sensory Evaluation

The sensory evaluation of guava herbal kefir and the control sample was conducted by a panel consisting of 10 semi-trained individuals who were all final year students pursuing their MSc degrees.

To evaluate the overall acceptance of the beverage, a 9-point Hedonic scale was utilized. This scale, as described by Amerine, 1965, ranges from 1 (dislike extremely) to 9 (like extremely). A group of 10 untrained tasters, consisting of both males and females aged 20-25 years, were asked to indicate their level of liking or disliking for each beverage based on taste, odor, and appearance. The tasters were students and staff from the Food and Nutrition Department at Bhagat Phool Singh Mahila Vishwavidyalaya, Khanpur Kalan, Sonipat. The samples, which were refrigerated at 10 °C, were served in clear plastic glasses with a volume of 50 ml. To ensure anonymity, the glasses were marked with random single-digit numbers. The mean score of ten panelists for each sensory characteristic was calculated.

2.5 Physio-chemical Analysis

Titrable Acidity, pH, total soluble solids, Alcohol content and carbon dioxide were determined.

The analysis of moisture content, ash, fat and protein was performed according to AOAC (2005).

2.5.1Titrable acidity and pH

10 gm well mixed juice was diluted to 250 ml with boiled water. Titration was done with 0.1 N NaOH, 0.3 ml phenolphthalein for each 100 ml of the solution to pink. End point was taken for
30 seconds.

pH was taken by using pH meter.

2.5.2 Total Soluble Solids

The process of extraction involves obtaining herbal water kefir drink and filtering it through a muslin cloth to acquire a transparent filtrate. To measure the filtrate, a small amount is placed on the prism of a digital refractometer.

The total soluble solids (TSS) can be measured using a refractometer, and the results are expressed in degrees Brix (°Brix). Degrees Brix (°Brix) is a measurement unit that indicates the concentration of sugar in a liquid solution. Each degree Brix represents 1 gram of sucrose dissolved in 100 grams of solution. This technique is widely utilized in the food sector to evaluate the sweetness and overall quality of fruits and juices.

3 Results and discussions

3.1 Sensory Evaluation of all Herbal kefir

The experiments were conducted to study the preparation, quality evaluation studies and sensory characteristics, which were determined for all eight fresh and stored samples of Herbal water kefir and plain water kefir. The sensory characteristics viz. color, taste, flavor, appearance and overall acceptability were done on 9point hedonic scales are presented in **Table 1**.

The samples were stored at +4 degree Celsius as given by Gökırmaklı,2024. The scale was arranged such that: Like extremely = 9, Like very much = 8, Like moderately = 7, Like slightly = 6, Neither like nor dislike = 5, Dislike slight = 4, Dislike moderately = 3, Dislike very much = 2, Dislike Extremely = 1. While scoring, the highest score (9) was assigned to the most preferred characteristic and least score (1) to the least desired characteristic. While this evaluation does not reflect actual consumer perception, it gives an idea of what attributes a good quality product should possess

Table 1: Sensory Parameters of guava Kefir Mocktails under Cool Temperature

Sample	Appearance	Taste	Smell	Texture	Overall Acceptability
Guava Kefir	7.7 ± 0.2^b	7.7 ± 0.2^b	7.7 ± 0.2^b	7.6 ± 0.1^b	7.675 ± 0.2^b

Values are Mean ± SD (n=3). SE = SD/√3. Different superscripts (a-d) indicate statistically significant differences ($p \leq 0.05$).

Appearance, Taste, and Smell all received identical scores of 7.7 ± 0.2^b, indicating uniform high acceptability. Texture was rated 7.6 ± 0.1^b, marginally lower but still within the same homogeneous significance group, implying that the beverage maintained a smooth and palatable consistency, possibly due to controlled fermentation

One-way ANOVA confirms no significant differences ($p > 0.05$) among sensory attributes, indicating uniform consumer appreciation across variables.

DMRT superscript '^{b}' denotes all attributes fall into the same homogeneous group, suggesting consistent perception with no standout outliers or rejection indicators.

3.2 Physicochemical Evaluation of Guava Kefir

Physicochemical characteristics of guava herbal drink which are selected after sensory evaluation and one commercially available plain water kefir has been explained. Total energy, ash, calcium, TSS, pH, titratable acidity, alcohol, and CO_2 content across kefir mocktails stored at room and cool temperatures are shown in table 2.

The data presented in Table 2 illustrates the impact of two different storage temperatures—cool and room conditions—on the physicochemical characteristics of guava-based kefir beverages. The sample that was kept at room temperature had a significantly higher energy content (53.06 ± 0.45 Kcal) than the sample that was kept in a cool environment (39.96 ± 0.14 Kcal). This difference could be attributed to improved metabolite yield and microbial fermentation. Compared to cool settings (2.82 ± 0.24%), room temperature conditions significantly increased ethanol production (3.58 ± 0.59%), highlighting the importance of temperature in promoting yeast metabolism and fermentation dynamics.

Under room conditions, the pH decreased somewhat (3.45 ± 0.57) compared to cool storage (3.55 ± 0.91), indicating increased acidogenesis. Titratable acidity, on the other hand, stayed consistent between the two treatments (4.36%), indicating buffering interactions between secondary metabolites and organic acids. More microbial respiration and effervescence were indicated by somewhat higher CO_2 levels (1.36 ± 0.25%) in samples kept at ambient temperature as compared to those kept cool (1.29 ± 0.31%). Calcium (mg), ash (%), and total soluble solids (TSS%) all remained constant regardless of storage temperature, suggesting that temperature has no effect on sugar retention and mineral solubility.

Table 2: Physicochemical Properties of Guava Kefir

Parameter	Guava (Cool)	Guava (Room)
Total Energy (Kcal)	39.96 ± 0.14	53.06 ± 0.45
Total Ash (%)	0.17 ± 0.92	0.17 ± 0.77
Calcium (mg)	40.0 ± 0.27	40.0 ± 0.81
Total Soluble Solids (%)	1.82 ± 0.95	1.82 ± 0.33
pH	3.55 ± 0.91	3.45 ± 0.57
Titratable Acidity (%)	4.36 ± 0.43	4.36 ± 0.44
Alcohol (%)	2.82 ± 0.24	3.58 ± 0.59
CO_2 (%)	1.29 ± 0.31	1.36 ± 0.25

physicochemical analysis

	Control sample	Control sample®	GUAVA	GUAVA
TSS(%)	2.45	2.45	1.82	1.82
PH	3.48	3.12	3.55	3.45
TITRABLE ACIDITY(%)	4.45	4.45	4.36	4.36
ALCOHOL(%)	3.15	4.12 Para	meter 2.82	3.58
■ Co2(%)	2.55	2.51	1.29	1.36

value 05

TSS(%) PHTITRABLE ACIDITY(%) ALCOHOL(%) Co2(%)

Figure 2: Comparative Physicochemical evaluation of two guava-based kefir variants

Figure 2 represent the comparative physicochemical evaluation of two guava-based kefir variants under distinct fermentation/storage conditions. Parameters assessed include Total Soluble Solids (TSS), pH, Titratable Acidity, Alcohol Content, and Carbon Dioxide (%).
The physicochemical profiles suggest that guava-based kefir offers moderate acidity, controlled ethanol yield, and lower carbonation, all of which may contribute positively to sensory balance and consumer acceptability.

3.3 Vitamin Composition of Guava kefir

A comparison of the vitamin contents in guava-based kefir beverages stored at room temperature and at a cold temperature is shown in **Table 3**. Standard analytical methods were used to quantify a total of 11 vitamins and the results are given in µg/mL along with their respective standard deviations.
The levels of vitamin A were somewhat greater at cool temperatures (9.60 ± 0.56 µg/mL) than at room temperature (9.10 ± 0.18 µg/mL), which may indicate oxidative breakdown at high temperatures.
In samples kept at lower temperatures, the vitamin B-complex group (B1 to B12) showed generally higher retention.

The cool-stored sample had a significantly higher vitamin B12 concentration (3.75 ± 0.19 µg/mL) than the room-stored sample (3.11 ± 0.62 µg/mL), which would indicate that cobalamin stability is sensitive to temperature.
At lower temperatures, vitamin B9 (folic acid) also showed improved stability (8.21 ± 0.65 µg/mL vs. 7.88 ± 0.91 µg/mL).
Antioxidant vitamin C, which is extremely labile, showed slightly higher concentrations in cool storage (93.00 ± 0.40 µg/mL) than at ambient temperature (92.00 ± 0.85 µg/mL), which is associated with lower thermal breakdown.
The fact that vitamin D and E levels stayed relatively stable under both conditions indicates that these fat-soluble vitamins are thermal resistant.

Table 3: Vitamin composition of guava kefir at cold temperature and room temperature

Vitamin (µg/mL)	**Guava (Room)**	**Guava (Cool)**
Vitamin A	9.10 ± 0.18	9.60 ± 0.56
Vitamin B1	4.11 ± 0.45	4.15 ± 0.26
Vitamin B2	5.13 ± 0.11	5.31 ± 0.60
Vitamin B3	6.77 ± 0.19	6.89 ± 0.74
Vitamin B5	8.12 ± 0.61	8.25 ± 0.26
Vitamin B6	3.11 ± 0.29	3.25 ± 0.53
Vitamin B9	7.88 ± 0.91	8.21 ± 0.65
Vitamin B12	3.11 ± 0.62	3.75 ± 0.19
Vitamin C	92.00 ± 0.85	93.00 ± 0.40
Vitamin D	2.14 ± 0.78	2.14 ± 0.66
Vitamin E	2.07 ± 0.23	2.15 ± 0.59

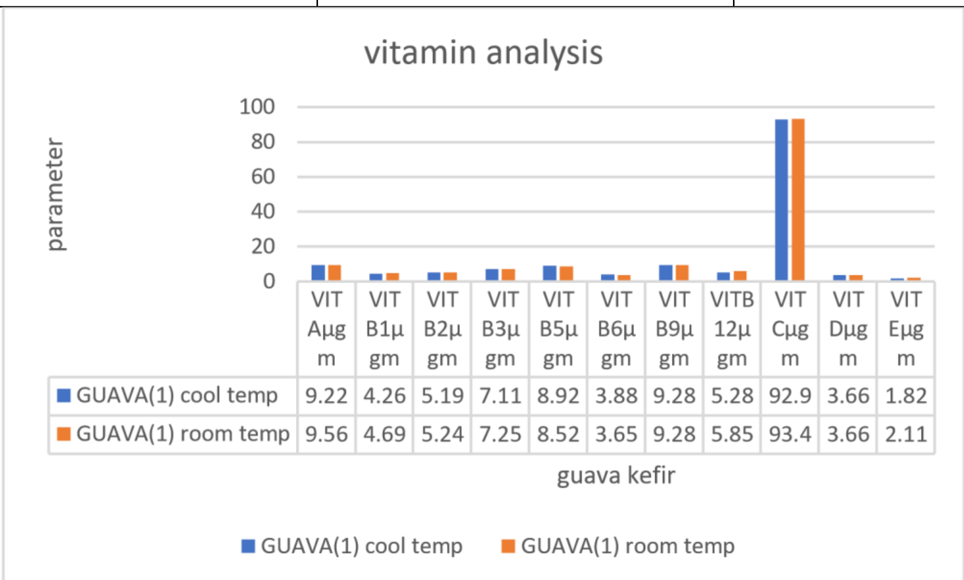

Figure 3: Comparative Vitamin Profile of Guava kefir Beverage under Room and Cool

Storage Conditions

Figure 3 illustrates the concentration of vitamins A, B-complex (B1–B12), C, D, and E of guava kefir stored at room and cool temperatures. Storage temperature significantly affects vitamin retention and degradation trends.

There was little negative impact from room temperature storage, and in certain circumstances, there was a slight vitamin boost, potentially as a result of microbial fermentation.

Heat-sensitive vitamins (such as B5, B6) were generally somewhat better preserved in cool storage, although the differences were not significant.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of three independent replicates.

All statistical tests were conducted at a 95 % confidence level, with $p < 0.05$ considered significant. One-way analysis of variance (ANOVA) was applied to evaluate differences among treatment means for each parameter. Whenever ANOVA indicated a significant F-value ($p \leq$ 0.05), pairwise comparisons were performed using:

- Tukey's Honest Significant Difference (HSD)
- Duncan's Multiple Range Test (DMRT)

Results

Clearly demonstrating the impact of guava leaves enrichment on the organoleptic properties of kefir beverages, the sensory study revealed statistically significant changes between appearance, taste, smell, texture, and overall acceptability (p 0.05).

The increased energy content during fermentation at room temperature may have resulted from increased microbial activity that converted carbohydrates into ethanol and other metabolites.

At room temperature, the alcohol level rose noticeably, indicating that temperature is a critical factor in boosting yeast metabolism in guava kefir.

Although titratable acidity stayed constant, maybe as a result of the buffering effects of other metabolites, pH decreased slightly, suggesting increased lactic acid generation in warmer settings.

The idea that room temperature promotes more effervescence and microbial respiration was supported by the rise in carbon dioxide levels.

Guava kefir was more resistant to physicochemical degradation with time. The bioactive phenolics in guava probably altered microbial metabolism, leading to moderate acidity and relatively stable TSS.

According to these results, guava-based kefir beverages' vitamin profile—especially that of water-soluble and heat-sensitive vitamins—is better preserved under cold storage circumstances. The implications for enhancing post-fermentation storage techniques to increase the nutritional value of functional beverages are significant.

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