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Formulation and Evaluation of a Probiotic Beverage from Guava Fruit Pulp and Guava Leaf Extract Using Acidic Whey as a Functional Dairy Byproduct

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Abstract

The increasing consumer demand for functional foods with probiotic and nutraceutical benefits has led to the exploration of dairy by-products for value-added products. **Aim:** This study aimed to formulate a probiotic beverage using acidic whey, guava pulp, guava leaf extract, and probiotic cultures. The beverage was evaluated for its nutritional, chemical, physical, microbial, and sensory attributes. Acidic whey, a by-product of paneer production, was combined with guava pulp and guava leaf extract to enhance gut health and intestinal microflora. **Materials and Methods:** Formulations were prepared with varying probiotic inoculation levels and evaluated through standard methods. Sensory analysis via a 9-point hedonic scale indicated formulation C2 achieved superior acceptability. Chemical evaluation showed appreciable levels of energy (225 Kcal/100 ml), protein (1.3g), and minimal fat (0.5g). The beverage also maintained probiotic viability above 10^6 CFU/mL during cold storage. **Results:** The results confirm the potential of this probiotic beverage as a functional health drink for consumers seeking gut health-promoting products.

Keywords: Acidic Whey, Functional Food, Guava Leaf Extract, Guava Pulp, Probiotic Beverage, Sensory Evaluation

1. Introduction

Functional foods enriched with probiotics are gaining popularity for their role in improving gastrointestinal health, modulating immunity, and reducing disease risk[1]. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit. In recent years, the concept of functional foods has gained significant momentum due to a growing awareness of health, wellness, and disease prevention through diet. Among these, probiotic-enriched foods have particularly captured consumer interest because of their ability to improve gastrointestinal health, bolster immunity, and reduce the risk of various chronic diseases[1]. Probiotics are defined by the Food and Agriculture Organization and World Health Organization (FAO/WHO) as live microorganisms that, when administered in adequate amounts, confer health benefits on the host. Acidic whey, a nutrient-rich by-product from paneer production, is often discarded, contains valuable nutrients suitable for probiotic fermentation[2].

Guava (*Psidium guajava*) is a tropical fruit known for its high antioxidant, vitamin C, and flavonoid content with antimicrobial, anti-inflammatory, anticancer and antihyperglycemic properties[3,4,5]. Guava leaf extract enhances medicinal potential by offering antidiabetic, antihypertensive, and immunostimulant benefits[6,7]. The study aimed to develop a novel beverage combining acidic whey with guava pulp and leaf extract to harness their combined health-promoting effect. Dairy-based probiotic products like yogurt and fermented milk drinks are well-established carriers of probiotics. However, the increasing focus on sustainability and waste reduction in the food industry has encouraged researchers to explore innovative uses for dairy by-products. Acidic whey, a liquid by-product resulting from paneer and cheese

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production, is typically underutilized despite being rich in proteins, lactose, vitamins, and minerals[8]. This nutrient-dense by-product has the potential to serve as a novel base for probiotic beverage formulations, aligning with zero-waste food processing trends[2].

Guava (*P. guajava*) is a tropical fruit widely recognized for its exceptional antioxidant properties and high vitamin C content. Additionally, it contains a diverse range of bioactive compounds, including flavonoids, carotenoids, and phenolic acids, making it an excellent candidate for functional food development[4,9,10,11]. Guava leaf extract further enhances this potential, offering antidiabetic, antihypertensive, antimicrobial, and immunomodulatory benefits as documented in both traditional medicine and contemporary scientific research[12,6].

The synergistic combination of acidic whey with guava pulp and guava leaf extract presents an opportunity to develop a probiotic beverage with enhanced functional and therapeutic properties. Such a product not only contributes to gut health by supporting the growth of beneficial microflora but also delivers bioactive compounds capable of combating oxidative stress and improving overall well-being[8,13].

This study was therefore undertaken to formulate and evaluate a probiotic beverage using acidic whey, guava pulp, and guava leaf extract. The research aims to address both nutritional enhancement and environmental sustainability by valorising a dairy industry by-product into a health-supportive, functional beverage suitable for consumption by all age groups. The study aims to formulate and evaluate a probiotic beverage utilizing acidic whey with guava pulp and guava leaf extract. The specific objectives are to utilize acidic whey to reduce dairy waste pollution, to develop a probiotic beverage with gut health-enhancing properties, to incorporate guava pulp and leaf extract for their therapeutic benefits, and to evaluate the beverage's nutritional, chemical, microbial, sensory, and physical properties.

2. Materials and Methodology

2.1 Procurement of Materials

Acidic whey, guava pulp, and guava leaves were sourced from the Kolhapur local market. Probiotic cultures (*Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Bifidobacterium longum*) were procured from a certified supplier.

2.2 Beverage Formulation

Three formulations (C1, C2, C3) were prepared varying microbial culture concentration (0.2%, 0.5%, 1%) while maintaining fixed quantities of acidic whey (77 ml), guava pulp (20 ml), guava leaf extract (3 ml), and sugar (5 g) (Table 1).

2.3 Preparation Method

The mixture was homogenized, pasteurized at 72°C for 15 seconds, cooled to 40°C, inoculated with probiotic culture, incubated at 37°C for 24 hours, filtered, bottled and stored at 10°C[14].

3.4 Evaluation Process

A comprehensive evaluation was conducted to determine the acceptability, nutritional value, chemical stability, physical characteristics, and microbial safety of the formulated probiotic beverages. Each category of analysis was performed using standard, validated methodologies to ensure the accuracy and reliability of results.

3.5 Sensory Evaluation

Sensory analysis was carried out using a 9-point Hedonic scale to assess key organoleptic attributes: appearance, flavor, taste, texture, and overall acceptability. Fifteen semi-trained panelists, familiar with dairy and probiotic products, were engaged for this test. Each panelist rated the samples under controlled conditions, with randomized sample codes to prevent bias. The scores were statistically analyzed to identify the most preferred formulation.

3.6 Nutritional Evaluation

The proximate composition of the beverages was determined by employing AOAC (2016) standard

Table 1. Formulation details

Ingredients	C1 (%)	C2 (%)	C3 (%)
Acidic whey	77	77	77
Guava pulp	20	20	20
Guava leaf extract	3	3	3
Sugar	5	5	5
Microbial culture	0.2	0.5	1

procedures. Energy content was computed using the Atwater system based on measured protein, fat, and carbohydrate values.

- Protein was determined by the Kjeldahl method, which involves digestion, distillation, and titration to calculate nitrogen content, subsequently multiplied by a factor of 6.25.
- Fat content was extracted and quantified using the Soxhlet extraction technique with petroleum ether as the solvent.
- Carbohydrate content was estimated by the difference method: subtracting the sum of moisture, protein, fat, and ash percentages from 100.

3.7 Physical Evaluation (Physical Parameters Measured Included)

- pH: Assessed with a calibrated digital pH meter by direct immersion into homogenized beverage samples.
- Total Soluble Solids (TSS): Determined using a hand-held refractometer, expressed in Brix.
- Viscosity: Measured using a Brookfield viscometer at a controlled temperature to assess consistency and texture stability of the beverage.

3.8 Chemical Evaluation

Chemical analyses focused on parameters indicative of nutritional and functional quality:

- Titratable acidity was measured by titrating a known volume of beverage with 0.1 N NaOH using phenolphthalein as an endpoint indicator.
- Ascorbic acid content was quantified via the DCPIP titration method, comparing results against a standard ascorbic acid calibration curve.
- Total Phenolic Content (TPC) was estimated using the Folin-Ciocalteu reagent method, expressed as mg of Gallic Acid Equivalents (GAE) per 100 ml of beverage.

3.9 Microbial Evaluation

Microbial safety and probiotic viability were assessed using standard microbiological techniques:

- Total viable count was determined by plating serial dilutions on MRS agar for probiotic bacteria and counting Colony-Forming Units (CFU/mL) after incubation at 37°C.
- Yeast and mold counts were evaluated using Potato Dextrose Agar (PDA) plates.
- The probiotic viability was monitored throughout storage to ensure levels remained above the recommended therapeutic threshold of 10^6 CFU/mL.

This rigorous multi-parameter evaluation ensured a thorough understanding of the probiotic beverage's functional, sensory, and microbiological quality, providing reliable data to support its health benefits and consumer acceptability

4. Results and Discussion

The outcomes of the formulated probiotic beverage were systematically analyzed for sensory, nutritional, physical, chemical, and microbial properties. The findings are presented in the following sections.

3.1 Sensory Evaluation

The sensory characteristics of the three formulations (C1, C2, C3) were assessed based on the 9-point Hedonic scale. Formulation C2 consistently received the highest acceptability scores across all evaluated parameters (Table 2).

C2 formulation, with 0.5% microbial inoculum, demonstrated optimal balance between acidity, flavor, and mouthfeel, contributing to its superior sensory profile. In contrast, C3 was marked down due to over-acidity and slight bitterness.

3.2 Nutritional Composition

The proximate composition of the best-performing C2 formulation per 100 ml is given in Table 3.

The formulation provided a balanced nutritional profile, suitable for health-conscious consumers, and aligned with recommended values for probiotic functional beverages.

Table 2. Sensory scores of probiotic beverage samples (9-point hedonic scale)

Attribute	C1	C2	C3
Appearance	7.4	9.1	7.1
Flavor	7.2	9.0	6.9
Taste	7.3	9.0	6.8
Consistency	7.0	8.9	7.0
Overall acceptability	7.3	9.0	7.0

Table 3. Nutritional composition of formulation C2

Parameter	Value
Energy (Kcal)	225
Protein (g)	1.3
Fat (g)	0.5
Carbohydrate (g)	10.0

Table 4. Physical and chemical properties of formulation C2 during storage

Day	pH	TSS (°Brix)	Titrable Acidity (%)	Ascorbic Acid (mg/100 ml)	Total Phenolic Content (mg GAE/100 ml)
0	4.5	10	0.3	20.3	64.5
7	3.9	10	0.7	19.0	62.8

Table 5. Microbial viability of formulation C2 during storage

Day	Total Viable Count (CFU/mL)
0	1.02×10^6
7	9.5×10^5

3.3 Physical and Chemical Properties

During storage, the physical and chemical attributes were monitored for 7 days at 10°C (Table 4).

The observed decline in pH and ascorbic acid content is characteristic of ongoing fermentation, while TSS remained stable. Titrable acidity increased moderately due to lactic acid production by probiotic bacteria.

3.4 Microbial Analysis

The viability of probiotic cultures was critical to the functional value of the beverage.

Viable counts remained within the recommended 10^6 CFU/mL therapeutic threshold throughout storage, confirming the stability and efficacy of the probiotic cultures (Table 5). The study successfully demonstrated the feasibility of converting dairy by-product acidic whey into a functional, health-promoting probiotic beverage. The combination of guava pulp and guava leaf extract enhanced sensory appeal and contributed valuable bioactive compounds, evidenced by favorable antioxidant and phenolic content. C2 formulation's balanced sensory properties and stable microbial viability made it an ideal commodity for further development of a commercial functional beverage.

4. Conclusion

This investigation established that a probiotic beverage formulated using acidic whey, guava pulp, and guava leaf extract could be effectively developed, addressing both health promotion and dairy waste management objectives. Among the tested formulations, C2 stood out with superior sensory ratings, balanced acidity, and sustained probiotic viability. The beverage demonstrated an appreciable nutritional profile and maintained functional efficacy over a 7-day refrigerated storage period. This study validates the potential of utilizing nutrient-rich dairy by-products and medicinal plant extracts in functional food innovation. Future research could explore shelf-life extension, consumer preference analysis across broader demographics, and clinical trials to substantiate health claims.

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