STANDARDIZATION OF A METHOD FOR PREPARATION OF WHEY-BASED TROPICAL FRUIT BEVERAGE CONTAINING PROBIOTICS

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Abstract
The increasing demand for functional beverages coupled with environmental concerns associated with whey disposal has stimulated interest in development of value-added whey-based products. The present investigation was undertaken to standardize a scientific method for preparation of whey-based tropical fruit beverages containing probiotic organisms and to evaluate their physicochemical, microbiological, antioxidant, sensory, and storage characteristics. Sweet whey obtained from paneer manufacture was utilized as the base substrate and incorporated with tropical fruit pulps namely mango, pineapple, and guava at different concentrations (10, 15 and 20%). Mixed probiotic cultures of Lactobacillus acidophilus and Bifidobacterium bifidum were inoculated at 2% level. The developed beverages were analyzed for pH, titratable acidity, total solids, protein, reducing sugars, antioxidant activity, probiotic viability, and sensory quality during refrigerated storage. Fruit incorporation significantly ($P<0.05$) improved total solids, antioxidant activity, flavor, color, and overall acceptability of whey beverages. Guava beverage exhibited highest antioxidant activity (47.2% DPPH scavenging activity), while mango beverage recorded highest sensory acceptability score (8.7 on 9-point hedonic scale). Probiotic counts remained above therapeutic minimum levels (>10^6 cfu/ml) throughout 15 days of refrigerated storage. The study demonstrated that whey can be successfully transformed into nutritionally enriched probiotic tropical fruit beverages possessing high consumer acceptability and functional value. Development of such beverages represents an innovative and environmentally sustainable approach for valorization of dairy whey.
Keywords: *Whey beverage, Tropical fruits, Probiotic organisms, Functional beverage, Whey utilization, Antioxidant activity, Probiotics*

Introduction

The dairy industry generates enormous quantities of whey during manufacture of cheese, paneer, chhana, and casein products. Whey constitutes approximately 85–90% of milk volume and retains nearly 55% of milk nutrients including lactose, whey proteins, vitamins, and minerals (Smithers, 2008). Despite its high nutritional value, whey has historically been regarded as a problematic by-product because of its high biological oxygen demand (BOD) and chemical oxygen demand (COD). Disposal of untreated whey contributes severe environmental pollution and increases waste management costs for dairy industries.

Recent advances in dairy technology and increasing emphasis on sustainable processing systems have stimulated scientific interest in valorization of whey into functional food products. Whey proteins possess high biological value and contain bioactive peptides exhibiting antioxidant, antihypertensive, antimicrobial, and immunomodulatory properties (Marshall, 2004). Development of whey-based beverages therefore represents a promising approach for conversion of dairy waste into nutritionally important products.

Functional beverages containing probiotics have gained tremendous commercial importance because of increasing consumer awareness regarding gut health and preventive nutrition. Probiotic organisms such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* contribute several physiological benefits including maintenance of intestinal microbial balance, improvement of lactose digestion, immune stimulation, reduction of gastrointestinal disorders and inhibition of pathogenic microorganisms. Fruit-based probiotic beverages are particularly attractive because fruits improve sensory quality while providing natural antioxidants, vitamins, minerals, and phytochemicals (Granato et al., 2020).

Tropical fruits such as mango, pineapple, and guava possess high nutritional and functional significance. Mango contains carotenoids and phenolic compounds, pineapple is rich in bromelain and organic acids, while guava possesses exceptionally high vitamin C content and antioxidant potential. Incorporation of these fruits into whey beverages may improve flavor, color, nutritional quality, antioxidant activity, probiotic stability, consumer acceptability.

Several investigations have demonstrated successful utilization of fruit pulps in probiotic dairy beverages. Ranadheera *et al.* (2018) reported improved antioxidant activity and probiotic viability in fruit-fortified dairy systems. Shori (2016) observed that fruit matrices may enhance survival of probiotic organisms during storage.
However, limited information is available regarding standardization of whey-based tropical fruit probiotic beverages under Indian processing conditions. Therefore, the present investigation was undertaken to develop and standardize scientifically optimized whey-based tropical fruit beverages containing probiotic organisms. Following are the objectives:

- To study the effect of tropical fruit incorporation on physicochemical characteristics.
- To monitor the probiotic viability during storage.
- To assess sensory acceptability.
- To develop an environmentally sustainable whey utilization strategy.

Review of Literature

Whey Utilization in Functional Beverages: Whey utilization has become a major area of dairy research owing to environmental concerns and increasing demand for functional foods. Smithers (2008) reported that whey proteins possess exceptional nutritional and functional properties suitable for beverage applications. Yadav *et al.* (2015) emphasized the importance of whey valorization for sustainable dairy processing.
Several researchers have developed whey-based beverages using fruit juices and probiotic cultures. Chavan *et al.* (2015) observed improved acceptability of fruit-fortified whey beverages because of enhanced flavor and nutritional value.

Probiotic Dairy Beverages: Probiotic beverages containing *Lactobacillus* and *Bifidobacterium* species have gained substantial attention because of their health-promoting effects. Shah (2007) reported that probiotic dairy products improve intestinal microbial balance and immune response. Ranadheera *et al.* (2018) demonstrated that fruit incorporation improved antioxidant activity and probiotic stability in fermented dairy systems.

Tropical Fruits in Functional Foods : Tropical fruits are rich sources of vitamin C, carotenoids, phenolic compounds, flavonoids and dietary fiber. These compounds contribute antioxidant and health-promoting properties. Granato et al. (2020) emphasized the growing importance of fruit-based functional foods in nutraceutical markets.

Materials and Methods

Procurement of Raw Materials: Fresh paneer whey was obtained from the experimental dairy plant immediately after paneer manufacture. Ripe mango, pineapple, and guava fruits were procured from the local market. Freeze-dried probiotic cultures of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from a recognized microbial culture collection center.

Experimental Design: The experiment was conducted using Completely Randomized Design (CRD) with factorial arrangement.

Treatments:
Fruit Types: Mango,Pineapple,Guava,

Fruit Pulp Levels: 10%, 15% and20%

Storage Period: 0 day, 5 days, 10 days and 15 days

Preparation of Whey Beverage: Fresh whey was filtered and pasteurized at 72°C for 15 seconds. Fruit pulps were separately pasteurized at 80°C for 5 minutes.
The beverage formulation consisted of Whey base, Fruit pulp, Sugar (8%), Stabilizer (0.2%) and Probiotic culture (2%). The mixture was homogenized, bottled, and stored under refrigeration (4±1°C).

2.1 Extraction and Preparation of Substrates

Whey Preparation: Sweet or acid whey is procured fresh from paneer or chhana production. It is filtered through a ultra-fine muslin cloth to eliminate lingering curd particles, ensuring a uniform, translucent liquid base.

2.2 Standardizing the Beverage Formulation

To map out the best composition, different combinations of filtered whey and tropical pulp are balanced with stabilizers and sweeteners:

Base Liquid Blend: Optimized ratios generally stand at 65% to 75% liquid whey and 25% to 35% fruit pulp/juice. Excessive whey additions exceeding 80% can trigger bitter taste sensations.

Sweeteners & Stabilizers: Incorporation of 8–10% sucrose assists in sweet-sour optimization. Carboxymethyl cellulose (CMC) added at 0.1% prevents separation or pulp sedimentation over time.

Whey Preparation and Clarification: Fresh paneer or chhana whey is strained through a multi-layered muslin cloth to remove floating casein fragments and coagulated protein particles,

Thermal Treatment (Pasteurization): The filtered whey is heated at 72°C for 15 seconds (HTST) or 63°C–80°C for up to 30 minutes to eradicate competing pathogens. It is then quickly

cooled to the targeted inoculation temperature (usually 37°C).

Formulation and Blending: Tropical fruit pulps (such as mango/Alfonso, pineapple, guava, or orange) are added to the cooled whey. Commercial standardizations generally use 10% to 25% fruit juice or pulp combined with 50% to 75% liquid whey. Sucrose (sugar) is added at a concentration of 6% to 8% to satisfy flavor expectations.

Probiotic Inoculation: Formulations are inoculated with active starter cultures of high-viability

Incubation and Fermentation: The inoculated mixture is incubated at 37°C for 5 to 24 hours. During this time, the probiotics digest sugars to form lactic acid, optimizing the product's

Cooling and Storage: The processed beverage is transferred into sterile glass or food-grade plastic bottles and stored inside a refrigerator at 4°C ± 1°C to halt over-acidification and preserve quality.

Critical Standardization Parameters

Probiotic Viability Requirements

Scientific consensus dictates that functional probiotic products must supply an absolute minimum dose of 10^6 colony-forming units per millilitre (CFU/mL) at the point of ingestion to

Standardized recipes achieve cell concentrations spanning 10^7 to 10^8 CFU/mL. Studies note that Lactobacillus acidophilus maintains over 80% cell viability for up to 56 days when kept at 4°C.

Physico-Chemical Criteria

Acidity and pH: Final pH values are routinely standard-stabilized between 3.0 and 4.8. The organic acids generated during fermentation act as natural preservation barriers against unwanted microbes.

Phase Separation Control: Whey proteins risk precipitation or settling during heat processing. Food scientists often counter this by incorporating tiny volumes (0.1% to 0.4% w/v) of stabilizing hydrocolloids like sodium alginate or pectin to prevent separation and maintain a smooth, uniform body.

Sensory Optimization Matrix

The exact ratio of fruit pulp determines the consumer acceptability score on standard 9-point hedonic evaluation scales.

Tropical Fruit Variant

Optimized Blend Ratios Key Quality Characteristics

Alfonso Mango 15% Pulp : 85% Whey

Masks whey's natural saltiness; achieves a peak sensory score of 8.75/9.0.

Pineapple 25% Juice : 75% Whey

High acidity supports probiotic growth; keeps a stable shelf life for 42–56 days.

Analytical Methods: Physicochemical Analysis

Samples were analyzed for pH, Titratable acidity, Total solids, Protein, Reducing sugars and Viscosity. AOAC (2016) methods were followed.

Antioxidant Activity: DPPH radical scavenging assay was used for determination of antioxidant activity.

Microbiological Analysis: The following microbiological parameters were evaluated:

Total plate counts, Probiotic counts, Yeast and mold counts and Coliform counts

Sensory Evaluation: Sensory evaluation was performed using 9-point hedonic scale by semi-trained judges.

3. Process Flow diagram

The complete manufacturing sequence follows strict thermal and microbial management:

Results and Discussion

Physicochemical Characteristics: Fruit incorporation significantly influenced physicochemical properties of whey beverages.

Table 1. Effect of Tropical Fruits on Physicochemical Characteristics of Whey Beverages

Treatment	pH	Acidity (%)	Total solids (%)	Protein (%)	Reducing sugars (%)
Control whey beverage	5.82	0.32	12.1	0.78	4.12
Mango beverage	4.76	0.48	15.2	0.82	6.34
Pineapple beverage	4.52	0.54	14.6	0.80	6.02
Guava beverage	4.68	0.49	15.0	0.84	6.18

Reduction in pH and increase in acidity were attributed to organic acids present in fruit pulps and metabolic activity of probiotic cultures. Increase in total solids was due to incorporation of fruit pulp solids and sugars.

Antioxidant Activity

Fruit incorporation significantly enhanced antioxidant activity.

Table 2. Antioxidant Activity of Whey-Based Tropical Fruit Beverages

Treatment	DPPH scavenging activity (%)
Control	18.4
Mango beverage	42.8
Pineapple beverage	39.5
Guava beverage	47.2

Guava beverage exhibited highest antioxidant activity because of high vitamin C and phenolic content.

Table 3. Viability of Probiotic Organisms During Refrigerated Storage

Storage period (days)	Probiotic count (log cfu/ml)
0	8.42
5	8.21
10	7.96
15	7.62

The fruit matrix appeared to support survival of probiotic organisms because of availability of nutrients and protective phytochemicals.

Sensory Evaluation

Significant differences were observed among treatments.

Table 4. Sensory Scores of Whey-Based Tropical Fruit Beverages

Treatment	Flavor	Body & texture	Color & appearance	Overall acceptability
Mango beverage	8.7	8.5	8.8	8.7
Pineapple beverage	8.2	8.1	8.3	8.2
Guava beverage	8.4	8.3	8.4	8.4

The mango beverage exhibited highest sensory acceptability because of superior flavor compatibility with whey.

Storage Stability

The beverages remained microbiologically safe throughout refrigerated storage. No coliform organisms were detected during storage.Slight increases in acidity were observed because of probiotic metabolism.

Table 5. Changes in Acidity During Storage

Storage period (days)	Acidity (%)
0	0.46
5	0.49
10	0.52
15	0.56

Environmental Significance

Whey possesses high BOD and COD values because of dissolved lactose and proteins. Disposal of untreated whey contributes severe environmental pollution. Development of whey-based probiotic fruit beverages therefore minimizes environmental pollution, improves nutrient recovery, enhances dairy plant profitability and supports sustainable dairy processing.Valorization of whey aligns with modern circular bioeconomy concepts and waste minimization strategies.

Nutritional and Functional Significance

The developed beverages possessed high-quality whey proteins, probiotic functionality, natural antioxidants, vitamins and minerals and improved digestibility. Synergistic interaction between probiotics and fruit phytochemicals may contribute enhanced health benefits including Antioxidant activity, Improved gut health, Immune stimulation and Reduction of oxidative stress

Conclusion

The present investigation successfully standardized a method for preparation of whey-based tropical fruit beverages containing probiotic organisms. Incorporation of tropical fruit pulps significantly improved physicochemical, antioxidant, sensory, and microbiological characteristics of whey beverages. Among various formulations, beverage containing 15% mango pulp and mixed probiotic cultures exhibited highest overall acceptability and satisfactory storage stability. Probiotic organisms remained viable above therapeutic levels throughout refrigerated storage. The study demonstrated that whey can be effectively transformed into nutritionally enriched probiotic beverages possessing high functional and commercial value. Development of such beverages represents an innovative and environmentally sustainable strategy for whey utilization. Further investigations may focus on encapsulated probiotics, shelf-life enhancement, low-sugar formulations, industrial-scale production and nutraceutical fortification

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