

Research article

Development of fermented beverage from whey and kinnow juice

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ABSTRACT

The dairy sector generates large quantities of whey as a residual by-product, which poses environmental challenges due to its elevated organic content. Converting this nutrient-rich effluent into functional beverages represents an eco-conscious strategy for resource valorization. This investigation focused on formulating a probiotic beverage by integrating whey with kinnow (*Citrus reticulata*) juice, a citrus fruit recognized for its high concentrations of vitamin C and essential micronutrients. The aim was to develop a fermented functional drink and systematically evaluate its physicochemical attributes, sensory appeal, microbial integrity, and storage performance. Six distinct formulations (T0, T1, T2, T3, T4, T5) were produced using varying proportions of whey and kinnow juice, with T0 (100% whey) designated as the control. Each blend was inoculated with *Lactobacillus acidophilus* at 1% (v/v) and subjected to fermentation at 37°C for a duration of 5 hours. Sensory analysis was carried out by a panel of 10 individuals using a 5-point hedonic scale. Among the treatments, T2 (70% whey, 30% kinnow juice) achieved the highest acceptance scores, including 4.23 for taste, 4.05 for flavor, 3.98 for appearance, 3.86 for sweetness, and 4.02 for overall preference. This formulation was characterized by 0.20% fat, 2.29% protein, 390 mg/100 ml glucose, and 44.23 mg/100 ml ascorbic acid. The initial pH and titratable acidity were measured at 4.43 and 0.57%, respectively. Over 21 days of storage at 4 ± 1°C, pH decreased to 4.29 while acidity rose to 0.73%. Total viable counts remained above 10⁶ CFU/ml, indicating stable microbial levels during the study period. These findings confirm the feasibility of T2 as a nutritionally beneficial and environmentally sustainable probiotic beverage derived from dairy by-products.

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1. INTRODUCTION

Whey is a greenish-yellow, semi-translucent liquid that separates from the coagulable part or curd and is one of the highly nutritious by-products obtained from the dairy industry producing cheese, chhana, and paneer (Kober, 2024). However, around 55% of the nutrients in milk are retained in whey. The main whey proteins are β -lactoglobulin and α -lactalbumin, which contain essential amino acids and branched-chain amino acids. It also contains leucine which can minimize muscle loss under increased protein degradation and stimulate muscle protein synthesis. Sulfur-containing amino acids, such as cysteine and methionine - precursors of glutathione, have shown anti-carcinogenic and antioxidant properties and enhance the immune function (Davoodi et al., 2016). Considering the substantial nutritional value of whey and the diverse array of bioactive compounds present in kinnow (*Citrus reticulata*), the integration of these two ingredients presents a viable strategy for formulating functional beverages with improved health-promoting properties.

Kinnow, which is consumed worldwide, constitutes a significant source of antioxidants, volatile compounds, vitamins, and minerals. Kinnows are extremely rich in vitamin C and also loaded with vitamin B complex and minerals like sodium, potassium, calcium, and copper. Kinnow contains about 2.5 times more calcium than other citrus fruits (Purewal et al., 2020). These compounds exhibit anti-inflammatory, antihypertensive, diuretic, analgesic, and anti-hyperlipidemic activities. Kinnow (*Citrus reticulata* \times *Citrus deliciosa*) is extensively grown across South Asian countries, with peak harvest occurring between December and March. Its widespread availability during this season, coupled with cost-effectiveness, renders it a suitable candidate for use in the development of functional beverages. The selection of kinnow over other citrus fruits is attributed to its high juice extraction efficiency, substantial vitamin C and calcium content, and a well-balanced sugar-to-acid ratio. These attributes contribute to both enhanced nutritional value and improved organoleptic properties when incorporated into dairy-based probiotic formulations. Furthermore,

kinnow exhibits good fermentative compatibility with *Lactobacillus* species and contains inherent antioxidants, which facilitate efficient fermentation and contribute to the microbiological stability of the final product during refrigerated storage.

However, Whey is being considered as one of the most polluting dietary by-products since it has high biochemical oxygen demand and chemical oxygen demand. If whey is discharged into water bodies, it reduces the dissolved oxygen, hampers biodegradability, and poses a major risk to aquatic life, the environment, and human health. Whey dumping on land creates severe pollution concerns for the surrounding environment by affecting the physicochemical characteristics of soil which results in decreased crop yields. A viable method of whey utilization may be beverage production. Fermented whey retains comparable nutritional properties to its unfermented counterpart; however, the fermentation process offers the added benefit of reducing lactose content (Jeličić et al., 2008). To obtain beverages with functional properties, they are fermented by probiotic bacteria strains with desirable properties and documented clinical effects. One of the most popular probiotics in the food manufacturing industry is *Lactobacillus acidophilus*. It can produce vitamins, bacteriocins (antibacterial chemicals), and antibiotic-like substances. It has also significant health benefits on the digestive and immune systems (Shukla et al., 2013). While a few studies have explored the incorporation of whey in fruit-based beverage formulations in Bangladesh, in-depth investigations focusing on its fermentation with regionally abundant citrus fruits like kinnow are notably lacking. The present study aims to bridge this research gap by formulating a probiotic beverage using whey and kinnow juice, with a thorough assessment of its nutritional composition, sensory attributes, and microbiological quality, thereby evaluating its feasibility for potential commercial-scale production. The present research aimed to develop a probiotic fermented beverage by utilizing different proportions of whey and kinnow juice, inoculated with *Lactobacillus acidophilus*. The resulting formulations were systematically analyzed for their physicochemical characteristics, sensory appeal,

and probiotic viability. Furthermore, the storage stability of the most preferred formulation was monitored over a 21-day period under refrigeration ($4 \pm 1^\circ\text{C}$) to evaluate its functional attributes and potential suitability for commercial application.

2. MATERIALS AND METHODS

Raw materials used for beverage preparation

Whey, kinnow juice, sugar, starter culture, and artificial flavoring agent were used as the raw material for the study.

Preparation of whey

Milk containing 3.5% fat and 8.5 % solids not fat (SNF) was heated in a stainless-steel vessel to 95°C for 5 to 10 minutes. The heated milk was then brought down to 70°C by immersion in a chilled water bath, accompanied by periodic stirring to achieve a consistent temperature distribution before initiating acid-mediated coagulation. Then the milk was coagulated using 4% acetic acid as a coagulant with continuous stirring. The ratio of milk and acetic acid was 10:1 to 20:1 (v/v). The liquid (whey) was filtered using muslin cloth. In order to achieve greater clarification of the whey and eliminate residual protein constituents, the filtrate was thermally treated by heating to 105°C at an adjusted pH of 4.6. This process facilitates the precipitation of thermolabile whey proteins, notably β -lactoglobulin and α -lactalbumin, which undergo denaturation and aggregation under elevated temperature and acidic conditions (Swaigood, 2003). The application of this heat treatment improves the optical clarity and microbiological stability of the whey by reducing turbidity and inactivating potential contaminants, while largely retaining its nutritional and functional bioactive components. The treated whey was subsequently re-filtered and maintained under refrigerated storage at $4 \pm 1^\circ\text{C}$ for further application.

Preparation of kinnow juice

Kinnows were collected from the local market and washed in running water. The outer covering of kinnow was peeled off and the segments were

separated. After the seeds and the white covering were removed from each of the segments, then the mixture was blended and strained through muslin cloth to obtain orange juice. The juice was stored at $4 \pm 1^\circ\text{C}$ until use.

Development of beverages

Six different types of beverages were prepared by mixing different blends of whey and kinnow juice. Each formulation was standardized to contain 8% added sucrose to maintain consistent sweetness across all samples. The control (100% whey) was marked as T_0 and samples with 65, 70, 75, and 80% levels of blending with whey were marked as T_1 , T_2 , T_3 , and T_4 . The proportion of kinnow juice in the samples T_1 , T_2 , T_3 and T_4 was 35%, 30%, 25% and 20%. A supplementary formulation, designated as T_5 , was developed using 99% whey and 1% artificial flavor (orange flavor) to function as a control for flavor comparison purposes.

The selection of whey-to-kinnow juice blending ratios was guided by initial sensory screening, which indicated that incorporating 25–35% kinnow juice provided an optimal sensory equilibrium, balancing the natural acidity of citrus with the mild flavor profile of whey. This blending range was consistent with previously published studies, which have reported that fruit juice incorporation within the 20–35% range can significantly improve both the sensory appeal and nutritional value of probiotic dairy-based beverages (Shukla et al., 2013).

Each beverage formulation was inoculated with 1% (v/v) of *Lactobacillus acidophilus* (1×10^8 CFU/mL), previously isolated and molecularly characterized through PCR analysis, and subsequently incubated at 37°C for 5 hours to promote fermentation and probiotic activity (Shukla et al., 2013). The experimental layout is presented in Table 1.

Sensory evaluation

The sensory attributes of the fermented beverage formulations, including color and appearance, taste, flavor, sweetness, and overall acceptability, were systematically assessed using a 5-point hedonic scale, where a score of 5 indicated "like

very much" and a score of 1 represented "dislike very much" (Watts et al., 1989). Sensory evaluation was conducted by a semi-trained panel comprising ten members selected from among postgraduate students and academic staff of the Dairy Science Laboratory, Chattogram Veterinary and Animal Sciences University. Panelists were chosen based on their familiarity with dairy-based products and prior exposure to

sensory evaluation protocols, ensuring consistency and reliability in scoring. Each formulation was prepared in triplicate to account for batch variability, and the mean score of the three replicates was calculated for each attribute as rated by individual panelists. This approach enhanced the statistical robustness of the sensory data and minimized subjective bias.

Table 1. Experimental layout

| Treatments | Whey (%) | Kinnow juice (%) | Artificial flavor (orange) | Sugar (%) | Culture (%) |
|----------------|----------|------------------|----------------------------|-----------|-------------|
| T ₀ | 100 | 0 | 0 | 8 | 1 |
| T ₁ | 65 | 35 | 0 | 8 | 1 |
| T ₂ | 70 | 30 | 0 | 8 | 1 |
| T ₃ | 75 | 25 | 0 | 8 | 1 |
| T ₄ | 80 | 20 | 0 | 8 | 1 |
| T ₅ | 99 | 0 | 1% | 8 | 1 |

Chemical analysis of developed beverages

All samples were analyzed for different parameters such as moisture, fat, protein, ascorbic acid, and glucose content as well as pH and titratable acidity. Moisture percentage was determined according to the AOAC (2000). The fat content was determined by the Gerber method. The protein content was determined by the Micro Kjeldahl method. The ascorbic acid content was determined by Dye method, and glucose content was determined by GOD-PAP method (Enzymatic Colorimetric Test for glucose method without deproteinization). The pH was recorded by using a digital pH meter (Systronics Digital pH meter). The acidity percentage was determined by titrating with 0.1N sodium hydroxide solution as described by AOAC (2000).

Microbiological analysis

Total viable count (TVC) (Log₁₀ CFU/ml) was performed as a microbiological assay in the selected blends by standard plate count method. In short, plate count agar and sterile saline of 0.9% NaCl (w/v) were prepared. The samples (1 ml) were transferred into sterile saline and each serial dilution, then put into the plate and poured agar (10-15 ml) and allowed for solidification.

After solidified, the plates were incubated at 32°C for 48 hours. The colonies were enumerated with a plate having 30-300 colonies. In all cases, duplicate-counting plates were prepared for appropriate dilutions.

Statistical analysis

All collected data were tabulated in MS Excel first and then analyzed by statistical analysis software R version 4.33. One-way ANOVA procedure and Kruskal-Wallis test was used to evaluate the treatment effect on parameters. The collected data were expressed as Mean±Standard error. The significance of differences between means was tested using Tukey's Honestly Significant Difference Test (Tukey's HSD) for one-way ANOVA and Bonferroni for Kruskal-Wallis test. Statistical significance was considered when $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Sensory Evaluation

The data of sensory evaluation of different whey-based beverages is presented in Table 2. As presented in Table 2, formulations T1 and T2 emerged as the most preferred among panelists based on average sensory scores. From these, T₂

was preferred on average for flavor, taste, color appearance, and overall acceptability. The panelists also appreciated T₄ and T₅ for the sweetness, but less for another parameter. The table confirms this and indicates a small variability among the scores for T₁, T₂, and T₃. Among the treatments, T₂ obtained the highest score in every parameter while T₀ had the lowest scores. This could suggest that the specific formulation used in T₂ strikes a good balance in terms of flavor profile, sweetness level, and

visual appeal. Sensory analysis revealed that the T₂ formulation attained the highest average ratings across all evaluated attributes, including color and appearance (3.98), taste (4.23), flavor (4.05), sweetness (3.86), and overall acceptability (4.02), reflecting its superior palatability among the tested samples. Consequently, T₂ was selected for subsequent physicochemical and microbiological characterization.

Table 2. Sensory analysis of developed whey-based fermented beverages

| Treatments | Color and appearance | Taste | Flavor | Sweetness | Overall acceptability |
|----------------|-------------------------|------------------------|-------------------------|-------------------------|------------------------|
| T ₀ | 1.78±0.04 ^b | 2.87±0.01 ^c | 2.37±0.01 ^b | 3.92±0.01 ^b | 2.75±0.02 ^f |
| T ₁ | 3.95±0.01 ^{ab} | 3.98±0.01 ^b | 4.02±0.01 ^{ab} | 3.67±0.01 ^d | 3.87±0.01 ^b |
| T ₂ | 3.98±0.01 ^a | 4.23±0.01 ^a | 4.05±0.01 ^a | 3.86±0.01 ^c | 4.02±0.01 ^a |
| T ₃ | 3.70±0.01 ^{ab} | 3.75±0.01 ^c | 3.76±0.01 ^{ab} | 3.87±0.01 ^{bc} | 3.65±0.01 ^c |
| T ₄ | 3.56±0.01 ^{ab} | 3.73±0.01 ^c | 3.48±0.01 ^{ab} | 4.08±0.01 ^a | 3.53±0.01 ^d |
| T ₅ | 2.26±0.01 ^{ab} | 3.42±0.01 ^d | 3.22±0.01 ^{ab} | 4.12±0.01 ^a | 3.28±0.04 ^e |
| P-value | 0.005 | <0.001 | 0.006 | <0.001 | <0.001 |

[^{a-f} Mean bearing different superscripts in a column differs significantly between treatments at *P<0.05 level of significance, Mean±Standard error, T₀=100w, T₁=65w:35KJ, T₂=70w:30KJ, T₃=75w:25KJ, T₄=80w:20KJ T₅=99w with 1% OF; w=whey, KJ=kinnow juice and OF=orange flavor]

These results highlight the beneficial impact of kinnow juice supplementation on the sensory profile and consumer acceptance of whey-based beverages, particularly when compared to the control (T₀), which contained only whey. The findings are in agreement with earlier studies indicating that the integration of citrus or tropical fruit juices can significantly enhance the sensory appeal of functional dairy beverages. For example, Islam et al. (2021) documented a marked improvement in consumer preference with the incremental addition of pineapple juice to whey-based drinks. Similarly, Ahmed et al. (2023) reported increased sensory scores and acceptability in whey beverages enriched with mango juice.

The alignment between current and previous research may be attributed to the inherent aromatic compounds and flavor-enhancing

properties of fruit juices, which are effective in masking the often undesirable sensory

characteristics of whey proteins. However, observed differences in sensory outcomes across various studies could stem from variations in fruit composition, particularly in terms of acidity, sugar content, and aromatic profile, as well as differences in regional taste preferences among sensory panelists. Notably, kinnow juice offers a distinct combination of tanginess and natural sweetness, potentially contributing a more balanced and appealing flavor compared to other fruits like pineapple or mango. This suggests that while the addition of fruit juice generally improves sensory quality, the extent and nature of enhancement are strongly influenced by the specific fruit used.

Table 3. Chemical analysis of developed whey-based fermented beverages

| Treatments | Moisture (%) | Fat (%) | Protein (%) | Glucose (mg/100ml) | Ascorbic acid (mg/100ml) | pH | Acidity % |
|----------------|------------------------|-----------|-------------------------|-------------------------|--------------------------|-------------------------|--------------------------|
| T ₀ | 86.4±0.03 ^a | 0.13±0.03 | 2.08±0.02 ^d | 60.1±1.71 ^b | 0.0±0.0 ^b | 4.73±0.13 ^a | 0.46±0.01 ^b |
| T ₁ | 85.8±0.05 ^c | 0.17±0.03 | 2.38±0.03 ^a | 430.0±4.23 ^a | 49.1±1.10 ^a | 4.33±0.03 ^d | 0.58±0.003 ^a |
| T ₂ | 85.6±0.02 ^c | 0.20±0.00 | 2.32±0.02 ^{ab} | 412±3.58 ^a | 46.8±0.43 ^a | 4.43±0.03 ^{cd} | 0.57±0.001 ^{ab} |
| T ₃ | 85.4±0.04 ^d | 0.17±0.03 | 2.21±0.02 ^{bc} | 398±3.28 ^a | 45.3±0.20 ^a | 4.47±0.03 ^{cd} | 0.57±0.004 ^{ab} |
| T ₄ | 86.0±0.03 ^b | 0.17±0.03 | 2.17±0.02 ^{cd} | 350±4.36 ^a | 43.3±0.38 ^a | 4.53±0.03 ^{bc} | 0.56±0.004 ^{ab} |
| T ₅ | 86.3±0.02 ^a | 0.13±0.03 | 2.07±0.02 ^d | 60.9±1.70 ^b | 0.0±0.0 ^b | 4.71±0.03 ^a | 0.48±0.005 ^{ab} |
| P-value | <0.001 | 0.58 | <0.001 | 0.005 | 0.006 | <0.001 | 0.007 |

[^{a-d} Mean bearing different superscripts in a column differs significantly between treatments at *P<0.05 level of significance, Mean±Standard error, T₀=100w, T₁=65w:35KJ, T₂=70w:30KJ, T₃=75w:25KJ, T₄=80w:20KJ T₅= 99w with 1% OF; w=whey, KJ=kinnow juice and OF=orange flavor]

Chemical analysis

In our study, the moisture percentages varied across treatments, with T₀ having the highest value and T₃ having the lowest (Table 3). The moisture content observed in whey-based beverages aligned with the general range reported in similar studies (Aly et al., 2019). Whey-based beverages typically had high moisture content due to the aqueous nature of whey, which ranges from 85% to 87%, consistent with the findings of our studies.

The amount of fat% seemed very similar but T₂ had the highest fat content (0.2%) whereas T₀ and T₅ held the lowest (0.13%). The fat content remained relatively consistent across treatments, indicating that the variations in treatment did not significantly impact fat levels. The results also followed the outcomes of Yasmin et al., (2015) who reported 0.2 % fat in whey-based drinks. The highest protein content was determined in T₁ (2.38%), and the lowest protein content was found in sample T₅ (2.07%). Begum et al. (2019) found around 2-2.5% protein in whey and watermelon beverages but Pareek et al. (2014) found lower protein in orange juice carbonated whey drinks which was around 1.1%.

Although a fixed amount of sugar was added to the beverages, the glucose content varied significantly among the different treatments; T₁ has the highest value (430 mg/100ml) and T₀ has the lowest (60.1 mg/100ml). This suggested that different processing or ingredient compositions lead to variations in glucose levels. Whey-fermented beverages typically contain a small amount of glucose, primarily derived from lactose present in whey (4.72±0.03 to 5.45 ± 0.31). During fermentation, lactose is converted into lactic acid by lactic acid bacteria, which may result in a decrease in glucose levels (Yasmin et al., 2015; Dharani et al., 2021).

Furthermore, perceived sweetness may be lower in samples like T₁ and T₂, even when the measured glucose content is higher. This is a known phenomenon in sensory science and food chemistry, often due to the interaction between taste perception, acidity, and other sensory cues. Citrus-based juices such as kinnow are inherently rich in organic acids, particularly citric acid, which contribute to increased acidity and a corresponding decrease in pH. Elevated acidity levels are known to diminish the perception of sweetness by modulating taste receptor responses, regardless of the actual glucose concentration present in the formulation.

The highest amount of pH value (4.73) was found in sample T₀, whereas T₁ had the lowest pH value (4.33). The acidity of treatment T₀ was the lowest (0.46), and treatment T₁ was the highest (0.58). These differences in pH and acidity levels can influence the taste, stability, and overall sensory attributes of the beverage. The results regarding pH and acidity of functional drinks in current exploration agreed with the findings of Yadav et al. (2010) (pH 4.47 ± 0.02 to 5.01 ± 0.01) and Yasmin et al. (2015) (pH 4.89 ± 0.25 to 4.96 ± 0.29). Pakseresht et al., (2017) found the acidity of different yogurt drinks was 0.45-0.47% which was quite similar to the present research findings.

Microbial analysis

In the microbiological test, T₀ had the lowest TVC (2.25 x 10⁷ CFU/ml), and treatment T₄ had the highest (4.73 x 10⁷ CFU/ml) (Table 4). The normal TVC of whey fermented beverage can vary depending on factors such as the fermentation process, ingredients used, and storage conditions. According to International standards, the total viable counts in a probiotic product must be at least 6 log CFU/ml at the time of consumption (Korbekandi et al., 2011). However, a typical range for TVC in fermented beverages like whey-based drinks is between 7 to 9 log colony-forming units per milliliter (CFU/ml) (El-Jeni et al., 2018).

Table 4. Microbial analysis of developed whey-based fermented beverages

| Parameters | TVC (CFU x10 ⁷ /ml) |
|----------------|--------------------------------|
| T ₀ | 2.25±0.09 ^b |
| T ₁ | 3.91±0.07 ^{ab} |
| T ₂ | 4.03±0.06 ^{ab} |
| T ₃ | 4.47±0.07 ^{ab} |
| T ₄ | 4.73±0.07 ^a |
| T ₅ | 2.29±0.05 ^b |
| P-value | 0.007 |

[^{a-b} Mean bearing different superscripts in a column differs significantly between treatments at *P<0.05 level of significance, TVC= Total Viable Count, CFU=Colony Forming Unit, Mean±Standard error, T₀=100w, T₁=65w:35KJ, T₂=70w:30KJ, T₃=75w:25KJ, T₄=80w:20KJ T₅= 99w with 1% OF; w=whey, KJ=kinnow juice and OF=orange flavor]

Shelf-life study

Any attempt to prepare a fruit product is incomplete if its storage stability is not properly studied. Based on sensory evaluation and nutrient content, T₂ was found as the best blend. Thus, the shelf life of the selected blend was assessed. The formulated fermented beverage was evaluated for pH, acidity, and TVC at 10-day intervals (Table 5).

Table 5. Effect of storage period on developed whey-based fermented beverage

| Parameters | pH | Acidity % | TVC (CFU x10 ⁷ /ml) |
|------------|-------------------------|-------------------------|--------------------------------|
| Day 0 | 4.43±0.03 ^a | 0.57±0.002 ^c | 4.03±0.06 ^a |
| Day 10 | 4.37±0.03 ^{ab} | 0.63±0.002 ^b | 3.69±0.10 ^{ab} |
| Day 20 | 4.29±0.03 ^b | 0.73±0.004 ^a | 3.40±0.08 ^b |
| P-value | 0.01 | <0.001 | 0.005 |

[^{a-c} Mean bearing different superscripts in a column differs significantly between treatments at *P<0.05 level of significance. Mean±Standard error, TVC= Total Viable Count, CFU=Colony Forming Unit]

The table showed mean values of pH of freshly prepared beverages was 4.43 and it was 4.29 for the beverages stored for 21 days. This indicated storage period had a significantly decreasing effect on the pH. A decline in pH during storage was observed which may be due to the action of citric acid and ascorbic acid on the sugar and protein component of the product. Production of organic acids and amino acids also leads to an

increase in acidity thereby a decrease in pH, whereas protein and dietary fiber could protect probiotics from the detrimental effect of pH as reported for mango-based beverages (Sikder et al., 2001). A similar trend of pH was reported by Naik et al. (2009) in the case of whey-based watermelon beverages. Das et al. (2015) also found a reduction in the pH values from 4.28 to 3.95 in case of whey-based orange beverages.

The lower pH value of the beverage certainly increases the storage stability of the final product by impeding the growth of undesirable microorganisms including coliforms (Uzeh et al., 2009).

The changes in the titratable acidity during storage are affected by recipe and treatment combinations. In the selected sample, initial titratable acidity was measured as 0.57 and showed a progressive increase after 21 days of storage under refrigeration. The mean value of titratable acidity was 0.73 after 20 days. The increase in titratable acidity of the formulated beverage could be due to the acidic nature of both orange juice and whey. Lactose and proteins are converted into lactic acid and amino acids leading to an increase in acidity and decrease in pH of beverages (Jaspreet et al., 2015). An increase in acidity from 0.28 to 0.34% for mango-based and 0.25 to 0.28% for jackfruit-based RTS (Ready-to-serve) beverages was reported by Krishnaveni et al., (2001). Similar findings were also observed in case of mango-based RTS (Sikder et al., 2001) and for whey-based watermelon beverages (Naik et al., 2009). Hence, pH and acidity are the two important factors co-related to each other. Kumar (2015) found a similar trend of acidity (0.546 to 0.870%) in the shelf-life study of probiotic beverages using whey and aloe vera juice.

The initial TVC of the beverage was 4.03×10^7 CFU/ml which decreased to 3.40×10^7 CFU/ml at refrigerated storage. Although the viability of *Lactobacillus acidophilus* population decreased, the viable count of the probiotic beverage did not fall below 10^6 CFU/ml. The decrease in viable count is due to the decrease in the pH of the medium and the accumulation of organic acid as a result of growth and fermentation. The beverage sample was observed for 21 days, so no data were taken then after. Our results are in confirmation by other researchers who also reported a decline in the total viable count of whey-based probiotic beverages stored at $4 \pm 1^\circ\text{C}$ (Priyanka and Anjali, 2017).

Therefore, the 21-day shelf life observed under refrigerated storage ($4 \pm 1^\circ\text{C}$), during which the beverage retained its physicochemical stability, probiotic viability, and sensory acceptability,

indicates its suitability for commercial application as a fresh probiotic product. This meets the Codex Alimentarius and FAO/WHO threshold of $\geq 10^6$ CFU/mL for probiotic efficacy. Moreover, the storage duration aligns with industry norms for lightly processed dairy-based functional beverages, typically ranging from 14 to 30 days, affirming its feasibility for short-term retail distribution, assuming proper cold chain management.

4. CONCLUSION

The findings of this study confirmed that a fermented functional beverage developed using a 70:30 blend of dairy whey and kinnow juice, supplemented with 1% *Lactobacillus acidophilus*, exhibited favorable sensory attributes, maintained probiotic viability above 10^6 CFU/mL, and demonstrated stable physicochemical properties throughout 21 days of refrigerated storage at $4 \pm 1^\circ\text{C}$. Among the tested formulations, sample T₂ achieved the highest sensory evaluations, notably scoring 4.23 for taste and 4.02 for overall acceptability, reflecting significant consumer appeal. These outcomes highlight the efficacy of whey-kinnow juice as a promising substrate for probiotic fermentation, facilitating both nutritional enrichment and sustainable utilization of dairy by-products. From an application perspective, the beverage complies with key industry and international probiotic standards, supporting its feasibility for limited-scale commercial production and its potential role in enhancing public health through functional food consumption. Nevertheless, the study's scope was constrained by the limited number of sensory evaluators and the absence of predictive shelf-life modeling techniques. Future investigations should consider optimizing packaging solutions, scaling up fermentation processes, and conducting in vivo assessments to substantiate the probiotic efficacy and consumer benefits of such beverages.

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