Journal of Material and Process Technologies 2 (2024) 100130

Journal of Material and Process Technologies

Available online at http://journal.aastu.edu.et/

Development and Characterization of a Functional Fermented Whey-Based Beverage Flavored with Beetroot and Ginger

Abdulkadir Omer Fara1, Habtamu Admassu [2, 3]*

[1]FDRE Vocational and Technical Training Institute, School of Graduate Studies, Faculty of Agro-Processing, Department of Dairy Processing Technology
[2]Department of Chemical Engineering, College of Engineering, Addis Ababa Science and Technology University, P.O.Box 16417, Addis Ababa, Ethiopia
[3]Center of Excellence for Biotechnology and Bioprocess, Addis Ababa Science and Technology University, P.O.Box 16417, Addis Ababa, Ethiopia

Article Information

Article history:6 October, 2025
Received: 10 November 2025
Received in revised form : 25 Nov. 2025
Accepted : 3 December 2025

Keywords:
acid whey,
Beetroot juice,
ginger juice,
flavor,
functional fermented beverage

Corresponding Author
Email: habtamu.admasu@aastu.edu.et
(Habtamu A.)

https://doi.org/10.69660/jmpt.v2i1.128

Abstract

Whey, a nutrient-rich by-product of the dairy industry, is often underutilized despite its high nutritional value, thus requires proper utilization through value-added products. The aim of this study was to develop and characterize a functional fermented whey-based beverage flavored with beetroot juice and ginger, and to evaluate its quality during storage. Sensory evaluation identified the optimal formulation, with the 80:20 whey-to-beetroot ratio (T04) showing the highest acceptability in terms of color, flavor, taste, and overall quality. The selected formulation also contained 1% ginger and 10% sugar. Nutritional analysis showed that the formulated beverage had higher protein (3.33%), lactose (4.67%), and total solids (9.50%) compared to the control (acid whey), while fat content remained low. During 14 days of refrigerated storage, physicochemical and microbiological changes were observed. The pH decreased while titratable acidity increased, indicating ongoing fermentation. Total soluble solids and reducing sugars increased over time, whereas total phenolic content and antioxidant activity showed a slight decline. Microbial counts, including total bacteria, yeast, and molds, increased during storage, reflecting microbial activity in the beverage. Overall, the study demonstrated that the whey–beetroot–ginger beverage possesses good sensory acceptability, improved nutritional composition, and functional properties, highlighting its potential as a value-added functional beverage for industrial application.

1. Introduction

Milk and dairy products play a critical role in global food and nutrition systems due to their high-quality protein, essential minerals, and bioactive compounds. However, the rapid expansion of dairy processing industries has also resulted in increased generation of by-products, particularly whey. Whey is the liquid fraction remaining after milk coagulation during cheese production and is rich in lactose, proteins, vitamins, and minerals. Despite its nutritional value, whey has historically been underutilized and often treated as waste, creating both economic losses and environmental challenges.

Globally, whey production is estimated to exceed 160 million tons annually, yet a large proportion is still not fully valorized into value-added products [1]. In many regions, approximately 40–50% of whey is discarded without proper treatment. Within the European Union, whey utilization has improved due to technological advancements; however, utilization patterns still show that about 45% is used in liquid form, 30% is processed into whey powder, 15% is converted into lactose and related derivatives, and the remaining fraction is used for whey protein concentrate production [2]. The environmental impact of whey disposal is significant because whey contains high organic loads characterized by elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which can negatively affect soil fertility and aquatic ecosystems if discharged untreated [3].

Recent research has increasingly focused on sustainable whey utilization through food product development rather than waste disposal. Fermentation technology has emerged as one of the most promising approaches for converting whey into functional food products with improved nutritional and sensory properties. Fermented whey-based beverages can reduce lactose content, improve digestibility, and enhance probiotic and antioxidant properties. Modern consumer trends also show growing demand for natural, clean-label, and health-promoting functional beverages rather than highly processed drinks [4]. Additionally, plant-based enrichment of dairy beverages is gaining attention due to its potential to improve bioactive content and sensory characteristics [5].

Functional food research has demonstrated the health-promoting potential of plant-derived bioactive compounds. Beetroot (Beta vulgaris L.) is particularly important due to its high content of betalains, phenolic compounds, folate, and essential micronutrients. These compounds contribute to antioxidant, anti-inflammatory, and cardioprotective effects. Beetroot pigments also serve as natural food colorants, reducing dependence on synthetic additives [6]. Similarly, ginger (Zingiber officinale Roscoe) is widely used as a spice and medicinal plant due to its bioactive constituents, including gingerols and shogaols, which exhibit antioxidants, antimicrobial, and anti-inflammatory activities [7]. The combination of dairy matrices with plant bioactives has been shown to improve both functional and sensory properties of beverages.

In Ethiopia, the dairy sector is growing gradually alongside increasing demand for animal-source foods. According to the Food and Agriculture Organization, Ethiopia produced approximately 4.58 million tons of milk in 2023, reflecting ongoing improvements in livestock productivity and dairy management systems. The Central Statistical Agency of Ethiopia [8] also reported expanding livestock and dairy production activities across different agroecological zones. However, despite this growth, dairy processing infrastructure remains limited, and most milk is consumed in raw or traditional forms. Consequently, large quantities of whey generated from small- and medium-scale dairy processing enterprises are underutilized or discarded.

The Ethiopian dairy industry faces challenges similar to global trends, but with greater urgency due to limited waste management infrastructure. In this context, valorizing whey into functional beverages presents a promising strategy to enhance resource efficiency while supporting national food security goals. Moreover, the development of value-added dairy products can generate additional income for smallholder farmers and dairy processors, while simultaneously mitigating environmental pollution associated with whey disposal.

Beetroot and ginger are widely cultivated and culturally accepted food crops in Ethiopia, making them suitable candidates for functional beverage development. Ethiopia is also one of the major producers of ginger in Africa, supported by favorable climatic and soil conditions. The integration of locally available plant materials with dairy by-products aligns with sustainable food system development and circular economy principles [9].

Despite growing interest in whey valorization and plant-based functional beverages, there remains limited understanding of the physicochemical interactions, functional stability, and sensory optimization of fermented whey-based beverages enriched with beetroot and ginger, particularly under Ethiopian processing conditions. Furthermore, there is a lack of integrated studies evaluating formulation, storage stability, and microbiological quality of such composite functional beverages using locally available raw materials. Developing such products could improve nutritional intake, provide natural antioxidant sources, enhance sensory acceptability, and create market opportunities for dairy-based functional foods. Moreover, fermented whey-based beverages enriched with plant bioactives may help address micronutrient deficiencies while promoting sustainable agro-processing.

Therefore, this study aimed to develop and characterize a flavored functional fermented whey-based beverage enriched with beetroot juice and ginger. The study further evaluated the physicochemical, nutritional, antioxidant, sensory, and microbiological properties of the developed beverage during storage. The findings are expected to contribute to sustainable dairy by-product utilization, functional food innovation, and improved nutritional food availability in Ethiopia.

2. Materials and methods

2.1. Study location and materials

The development of the flavored functional fermented whey-based beverage was conducted at the Agro-Food Processing and Dairy Processing Laboratory of TVTI, Ethiopia. Additional biochemical and microbiological analyses were performed at the biotechnology laboratory of Addis Ababa Science and Technology University.

Fresh and mature beetroot and ginger were purchased from local markets in Addis Ababa, Yeka Sub-City. Standard milk was obtained from Shola/Lame Dairy Enterprise and transported in refrigerated ice boxes to maintain product quality. Starter cultures containing Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus were used for fermentation due to their well-documented roles in dairy fermentation and probiotic beverage production [4].

2.2. Preparation for acid whey

Acid whey was prepared following modified dairy processing procedures described by De [10]. Standardized milk was heated to 80–95° C for 10 minutes, followed by the addition of 2% citric acid to reach the isoelectric point of casein (pH ≈ 4.6). The curd was separated using muslin cloth filtration. The collected whey was cooled to 42 ± 1 °C and inoculated with 2% starter culture. Fermentation was carried out at 42 ± 1 °C for approximately 4 hours in a temperature-controlled incubator (or water bath) until the pH reached 3.1–3.8. Fermentation-based whey processing has been widely reported as an effective method for improving nutritional and sensory properties of dairy beverages [11].

2.3. Preparation of beetroot and ginger extracts

Beetroot was washed, sorted, peeled, cut into small pieces, and blended with water at a 2:1 beetroot-to-water ratio. The juice was filtered and heat-treated at 75°C for 30 seconds to reduce microbial contamination. Thermal treatment of vegetable juices is commonly used to maintain microbial safety while preserving bioactive compounds [12].

Ginger was washed, peeled, chopped, and blended with water at a 1:1 ratio. The juice was filtered and stored at 5 ± 1°C until use. Ginger bioactive compounds such as gingerols and shogaols are known to possess antioxidants and antimicrobial properties [13].

2.4. Beverage formulation

Seven formulations were prepared with different whey-to-beetroot ratios:

- T00 (100% whey – control)
- T01 (65% whey: 35% beetroot)
- T02 (70% whey: 30% beetroot)
- T03 (75% whey: 25% beetroot)
- T04 (80% whey: 20% beetroot)
- T05 (85% whey: 15% beetroot)
- T06 (90% whey: 10% beetroot)

All formulations contained 1% ginger and 10% sugar. Sugar addition was used to improve sensory acceptance and act as a mild preservative by reducing water activity [14].

Similar blending approaches have been reported in functional beverage development studies targeting consumer acceptability [15].

2.5. Sensory evaluation

Sensory evaluation was performed using a nine-point hedonic scale ranging from “like extremely” to “dislike extremely” following methods described by Sand and Narayanan [16]. Ten semi-trained panelists evaluated color, taste, flavor, and overall acceptability as this approach is consistent with standard practices in product development studies.

2.6. Storage stability study

The optimized beverage (80% whey, 20% beetroot, 1% ginger, 10% sugar) was packaged in sterile glass bottles and stored at 4 ± 1°C for 14 days based on practical, microbiological, and physicochemical considerations. Quality parameters were evaluated at 0, 7, and 14 days. Cold storage preservation is commonly applied in fermented dairy beverages to slow microbial growth and biochemical degradation [17].

2.7. Physicochemical Analysis

2.7.1. pH Measurement

pH was measured using a calibrated digital pH meter following AOAC [18] protocols.

2.7.2. Titratable Acidity

Titratable acidity was determined by titrating samples with 0.1 N NaOH and expressed as lactic acid percentage using standard analytical procedures [19].

$$\text{lactic acidity}\ \% = \frac{(0.1\ \text{N NaOH} \times \text{vol. of NaOH(in liter)} \times 90.8) \times 100}{\text{Weight of sample}}$$

Where, the molecular weight of lactic acid is (90.8 g/mol).

2.7.3. Total Soluble Solids

Total soluble solids were measured using a hand refractometer [20].

2.7.4. Protein, Fat, and Lactose

Protein, fat, and lactose were determined using a lactoscan analyzer as described by Ali et al. [21].

2.7.5. Total Solids

Total solids were determined using the oven drying method at 105°C until constant weight was achieved [22].

$$\text{Total solids} = W2/W1 \times 100$$

Where: W 1 = the initial weight of the sample; W 2 = the final weight of the sample.

2.8. Determination of bioactive compounds

2.8.1. Reducing sugars

Reducing sugar content was determined by using the DNS method according to Krivorotova and Sereikaite [23]. Absorbance was measured at 540 nm using a UV-Vis spectrophotometer.

2.8.2. Total Phenols

Total phenolic content was determined using the Folin–Ciocalteu method and expressed as gallic acid equivalents [24].

2.8.3. Antioxidant Activity

Antioxidant activity was evaluated using the DPPH radical scavenging assay according to Blois method modifications [25]. Functional antioxidant analysis is commonly used to evaluate health-promoting properties of plant-enriched dairy beverages [26]. The percentage of radical scavenging activity was calculated from the formula:

2.9. Microbiological Analysis

2.9.1. Sample Preparation

Aseptically, 10 mL (or 10 g) of the sample was transferred into 90 mL of sterile diluent (e.g., 0.1% peptone water), yielding an initial 10^{-1} dilution. The mixture was homogenized using a vortex mixer or stomacher for 1–2 minutes to ensure uniform distribution of microorganisms.

2.9.2. Serial Dilution and Plating

Microbial quality was assessed using standard serial dilution techniques [27]. Microbial enumeration was performed using the spread plate or pour plate method. Plating volumes of 0.1 mL (for spread plate) or 1 mL (for pour plate) of appropriate dilutions were used. Each selected dilution was plated in duplicate (or triplicate) to improve accuracy. Plates were prepared using selective or non-selective media depending on the target microorganisms, such as:

Plate Count Agar (PCA) for total viable count

Potato Dextrose Agar (PDA) for yeasts and molds

2.9.3. Total Bacterial Count

Samples were plated on nutrient agar and incubated at 37°C for 24–48 hours.

2.9.4. Yeast and Mold Count

Yeast and mold counts were determined using potato dextrose agar adjusted to pH 3.5 using tartaric acid and incubated at 25°C for 3–5 days.

2.10. Statistical Analysis

Experimental data were analyzed using SAS software version 9.4. One-way ANOVA was used to determine treatment effects, and Tukey’s test was applied at $p < 0.05$ significance level [28].

3. Results and discussion

3.1. Nutritional Characteristics of Acid whey

The nutritional characteristics of acid whey (protein, lactose, fat, total solids, pH, and total soluble solids) were studied. As shown in Table 1, the acid whey contained 2.0% protein, 4.67% lactose, 0.15% fat, 6.7% total solids, a pH of 3.34, and 6.0% total soluble solids. The protein content of this acid whey was found to be almost twice as high as reported by the FAO in 2013 and was in line with the findings of Ali, M. et al. [21], who reported a protein content of 2.6% in acid whey. The variance in reported protein content may be attributed to differences in the contributing labs and the impact of enzymatic production during fermentation. The fat content of the acid whey was determined to be 0.15%, falling within the range of 0.05 to

0.2% as reported in various literature. Variations in fat content may be due to differences in milk type, whey separation methods, heat treatment processes, and fermentation times. The pH of the acid whey was measured at 3.34, within the range of 3.0 to 4.6 as reported in various literature. The disparity in pH values may be attributed to differences in milk type, treatment methods, coagulants used, and fermentation techniques. In this study, the acid whey resulted from milk treated with citric coagulant and a lactic acid bacteria starter used for fermentation. The use of lactic acid bacteria as a starter culture is known to produce organic acids resulting in a decrease in pH. Additionally, the values of total solids, lactose, and total soluble solid contents of the acid whey are consistent with typical acid whey values reported by the FAO in 2013 and align with findings from other researchers [29].

Table 1. Nutritional analysis of acid whey

Protein	2±0.20
Lactose	4.67±0.15
Total Solid	6.7±0.36
Fat	0.15±0.02
Tss	6.00±0.29
pH	3.34±0.03

All values presented are means ±SD triplicate (n =3).

3.2. Physicochemical properties of beetroot juice

The physicochemical properties of beetroot juice were analyzed, including total soluble solids, reducing sugar, and pH. The results are shown in Table 2. The pH and total soluble solids values were found to be 5.21±0.026 and 8.00±0.50, respectively. Additionally, reducing sugar was found to be 3.71±0.29. These findings are consistent with previous studies by Ali, M. et al. [21], who reported TSS and pH in beetroot juice as 7.8 and 5.57, and by Kale, et al. [30], who also reported similar results with TSS, reducing sugar, and pH in beetroot juice at 9.0 brix, 4.0 mg/ml, and 6.3, respectively. It was noted that the total dissolved solids content is significantly affected by the combined influence of maturation stages and growing conditions [31].

Table2. Physicochemical analysis of beetroot juice

Total soluble solid	pH	Reducing sugar
8.00±0.50	5.21±0.03	3.71±0.29

All values presented are means ±SD triplicate (n =3).

3.3. Selection of better whey–beetroot–ginger combination beverage

3.3.1. Sensory Characteristics of Formulated Beverages

Sensory analysis remains a standard tool for optimization of functional food formulations before physicochemical characterization [32]. Sensory evaluation is a critical step in functional food product development because consumer acceptance determines market potential of new food products [33]. In this study, sensory scores increased with the addition of beetroot juice to fermented whey up to a certain level, after which scores gradually declined. Among all treatments, T04 (80% whey, 20% beetroot, 1% ginger, and 10% sugar) received the highest acceptability scores.

The improvement in sensory quality with moderate beetroot addition may be attributed to the natural sweetness, attractive red coloration from betalain pigments, and improved flavor balance. Similar findings were reported in whey-based functional beverages where moderate fruit or vegetable inclusion enhanced consumer preference [34].

3.3.2. Color, flavor, taste, and overall acceptability

Color is a major determinant of consumer choice in plant-dairy blended beverages. In this study, color scores ranged from 5.90 to 7.70, with T04 showing significantly higher preference ($P < 0.001$). The superior color acceptability of T04 can be attributed to optimal dilution of beetroot pigments, which provided an appealing natural red hue without being overly intense.

Flavor scores followed a similar trend, ranging from 6.10 to 7.60. The addition of ginger has likely contributed to improved aroma and masking of whey’s characteristic flavor. Ginger bioactive compounds such as gingerols contribute not only to flavor but also to antimicrobial and antioxidant properties [13].

Taste scores also increased with moderate beetroot inclusion, with T04 recording the highest score (7.90). This suggests that balanced blending improves sweetness-acidity balance, which is essential in fermented dairy beverages.

Overall acceptability scores confirmed T04 as the most preferred formulation. These findings align with studies showing that moderate plant juice incorporation improves sensory quality in dairy-based functional beverages due to improved flavor complexity and natural color enhancement [35]. Based on sensory optimization results, T04 was selected for further physicochemical and storage stability analysis.

Table 3. The sensory scores for selection of best formulated a flavored functional fermented whey beverage blended with beetroot and ginger (Mean ± SD).

Product sample	Color	Flavour	Taste	Overall acceptability
T00	5.90±0.18^{b}	6.10±0.10^{b}	6.10±0.23^{c}	6.03±0.07^{d}
T01	6.90±0.31^{ab}	6.60±0.34^{ab}	6.20±0.29^{bc}	6.57±0.20^{c}
T02	7.10±0.23^{a}	6.70±0.34^{ab}	7.20±0.29^{ab}	7.00±0.15^{bc}
T03	7.20±0.25^{a}	7.10±0.23^{ab}	7.00±0.26^{abc}	7.10±0.06^{b}
T04	7.70±0.26^{a}	7.60±0.31^{a}	7.90±0.28^{a}	7.73±0.09^{a}
T05	7.10±0.23^{a}	7.00±0.26^{ab}	7.00±0.21^{abc}	7.03±0.03^{bc}
T06	7.00±0.26^{a}	7.00±0.30^{ab}	7.00±0.26^{abc}	7.00±0.00^{bc}
P-value	0.0004	0.0132	0.0001	0.0001
CD-@ 0.05	1.061	1.173	1.099	0.5198
CV%	11.12	12.53	11.67	2.69

a-d All values presented are means ±SD (n = 10).
Means with the same superscript letters within a column are not significantly different(p>0.05).
where: - Treatment combination
T00 = 100% Whey, T01 = 65% W, 35%B, T02 = 70% W, 30% B, T03 = 75% W, 25% B, T04 = 80% W, 20% B, T05 = 85% W, 15%B, and T06 = 90% W, 10%B

3.4. Physicochemical properties of formulated beverage

3.4.1. total soluble solids (TSS)

Total soluble solids are important indicators of sweetness, shelf stability, and flavor intensity in beverage products. TSS increased with higher beetroot juice inclusion, ranged from 6.00 to 11.50 °Brix. The observed increase in total soluble solids (TSS) with increasing beetroot juice incorporation is attributed to the high content of soluble sugars (sucrose, glucose, and fructose), organic acids, and other water-soluble constituents present in beetroot. As the proportion of beetroot juice increased, these components contributed cumulatively to the overall soluble solid content, resulting in higher °Brix values compared to formulations with higher whey proportions.

The highest TSS was observed in T01, while T04 maintained moderate TSS levels that were considered optimal for sensory acceptability. Similar trends were reported in whey–fruit beverages where plant juice addition increased soluble solids concentration [36].

3.4.2. pH values

The pH values of the beverages ranged from 3.34 to 4.75. A slight increase in pH was observed with higher beetroot juice incorporation, likely due to dilution of organic acids in the fermented whey and the comparatively lower acid contribution from beetroot juice. Maintaining low pH is important for microbial safety and product shelf life. Fermented dairy beverages typically exhibit acidic pH due to lactic acid production by starter cultures such as Lactobacillus and Streptococcus species [17].

Table 4. Physicochemical properties of formulated whey beverage.

Product Sample	Response Variable	
	Total Soluble Solid	pH
T00	6.00 ± 1.00^{d}	3.34 ± 0.03^{f}
T01	11.50 ± 0.50^{a}	4.75 ± 0.22^{a}
T02	10.83 ± 0.29^{a}	4.60 ± 0.14^{ab}
T03	10.50 ± 0.50^{ab}	4.32 ± 0.21^{bc}
T04	10.17 ± 0.29^{abc}	4.06 ± 0.02^{dc}
T05	9.00 ± 1.00^{bc}	3.82 ± 0.19^{de}
T06	8.50 ± 0.50^{c}	3.56 ± 0.16^{ef}
P-VALUE	0.0001	0.0001
CD-@ 0.05	1.7997	0.4324
CV%	6.8	3.8

a-f All values presented are means ±SD triplicate (n =3).
Means with the same superscript letters within a column are not significantly different (p>0.05).

3.5. Nutritional composition of formulated beverage

The nutritional composition of the developed beverage improved compared to acid whey alone.

3.5.1. Protein content

Protein content increased from 2.00% in acid whey to 3.33% in the formulated beverage. This increase may be attributed mainly to concentration effects during processing and possible contributions from microbial biomass generated during fermentation, rather than direct protein contribution from beetroot juice. Similar trends of apparent protein enrichment have been reported in fermented plant–dairy blended beverages due to compositional changes and solids redistribution during fermentation and formulation [37].

3.5.2. Lactose content

Lactose content slightly decreased in the formulated beverage. This reduction is beneficial because it improves digestibility for lactose-sensitive consumers.

Fermentation converts lactose into lactic acid, improving flavor and shelf stability [38].

3.5.3. Total solid and fat content

Total solids increased significantly due to the addition of plant extracts. Fat content remained low and statistically non-significant, which is desirable for health-conscious consumers. Cheese whey typically contains 5–7% total solids composed mainly of lactose, minerals, and water-soluble nutrients [39].

Table 5. Nutritional analysis of Acid whey and formulated beverage.

Response variable	Sample code T00 (acid whey)	T04	P-value	CD @0.05	CV%
Protein	2.00 ± 0.20^{a}	3.33 ± 0.30^{b}	0.0034	0.578	9.62
Lactose	4.67 ± 0.15^{a}	4.03 ± 0.55^{a}	0.1274	0.9162	9.29
Total Solid	6.7 ± 0.36^{b}	9.50 ± 0.56^{a}	0.0019	1.0633	5.79
Fat	0.15 ± 0.02^{a}	0.14 ± 0.02^{a}	0.4107	0.0403	12.13

a- b All values presented are means ±SD triplicate (n =3).
Means with the same superscript letters within a column are not significantly different (p>0.05)

3.6. Storage stability study

3.6.1. Protein and lactose changes during storage

Protein content decreased gradually during storage due to proteolytic activity of lactic acid bacteria, which hydrolysed proteins into amino acids [40]. Lactose content also declined slightly due to continued fermentation activity during storage.

3.6.2. pH and titratable acidity

A significant decline in pH was observed during storage (4.06 to 3.12), while titratable acidity increased (0.28% to 0.44%). This trend is typical in fermented dairy beverages due to continuous lactic acid production during storage [41]. Lower pH improves microbial safety but may affect flavor intensity.

3.6.3. Total soluble solids and reducing sugar

TSS and reducing sugar increased during storage. This may be due to hydrolysis of complex carbohydrates into simple sugars. Similar results were reported in herbal whey beverages and fruit-based RTS beverages [42].

3.6.4. Total phenols and antioxidant activity

Total phenolic content (TPC) and antioxidant activity showed a slight decline during storage. This reduction may be attributed to the oxidative degradation of phenolic compounds, including enzymatic oxidation (e.g., polyphenol oxidase activity) and non-enzymatic reactions such as exposure to oxygen and light. In addition, interactions between phenolic compounds and proteins in the beverage matrix may lead to the formation of insoluble complexes, thereby reducing extractable phenolics. Despite this decline, the beverage retained considerable antioxidant activity, which can be associated with the stability of beetroot-derived betalains and the presence of ginger-derived phenolic compounds, which contribute to the overall antioxidant potential [26].

Table 6. Physicochemical analysis of Formulated beverage during storage at 4 ºC for 14days.

Parameter	Storage period (days)			p-value	CD @0.05	CV%
	0 days	7 days	14 days			
Protein	3.33±0.306ª	2.80±0.200ªᵇ	2.30±0.200ᵇ	0.0056	0.6022	8.6
Lactose	4.033±0.551ª	3.53±0.252ª	3.27±0.252ª	0.115	0.9485	10.5
TSS	10.17±0.289ᵇ	11.33±0.577ªᵇ	12.00±1.000ª	0.0444	1.7215	6.2
pH	4.06±0.015ª	3.37±0.010ᵇ	3.12±0.015ᶜ	0.0001	0.0494	0.6
DPPH	30.39±2.05ª	22.75±2.00ᵇ	22.08±2.90ᵇ	0.009	5.892	9.4
TA	0.28±0.020ᵇ	0.35±0.030ᵇ	0.44±0.040ª	0.003	0.0783	8.8
Total Phenol	46.36±0.076ª	45.12±0.009ª	43.25±0.984ᵇ	0.002	1.4279	1.3
Recuing sugar	5.46±0.031ᶜ	5.75±0.109ᵇ	7.57±0.112ª	0.0001	0.2305	1.5

a- c All values psesented are means ±SD triplicate (n =3).
Means with the same superscript letters within a Row are not significantly different (p>0.05)

3.7. Microbial Quality

3.7.1. Total Bacterial Count

Total bacterial counts increased but remained within FAO/WHO recommended probiotic levels (10^6–10^9 CFU/mL). This indicates that the beverage retained probiotic potential during storage. The growth of lactic acid bacteria may also be responsible for antimicrobial effects due to production of bacteriocins and organic acids [43].

Table 7. Microbial growth of formulated beverages during storage at 4 ºC for 14 days.

Microbial load		
Storage period (days)	Standard plate count CFU/mL	Yeast and mold CFU/ml
0 day	$3.89±0.306^{b}×10^{-7}$	NILL
7 day	$7.33±0.611^{a} × 10^{-7}$	$1.90±0.10^{b} ×10^{-5}$
14 day	$9.13±1.097^{a} × 10^{-7}$	$3.80±0.30^{a}×10^{-5}$
CD@0.05	1.87	0.5069
CV%	11	7.85
FAO limits	< 9	<5

a-b All values presented are means ±SD triplicate (n =3).
Means with the same superscript letters within a column are not significantly different (p>0.05)

3.7.2. Yeast and Mold Growth

Yeast and mold were initially absent but increased slightly during storage. The low pH and antimicrobial properties of ginger may have inhibited fungal growth in early storage days. Similar microbial trends were reported in fermented dairy beverages and probiotic functional drinks [44].

Conclusions

The study demonstrated that whey can be successfully converted into a functional fermented beverage using beetroot and ginger. The optimized formulation (T04) provided the best sensory acceptability, nutritional improvement, and acceptable storage stability. The results support the potential of whey valorization in developing countries as a strategy for sustainable food production, waste reduction, and nutrition enhancement.

4. References

1) Abdo, A. M., Mohamed, S. S., & Hassan, A. A. [21]. Development of antioxidant-rich whey beverages fortified with beetroot peel extract. Foods, 11(24), 4012.
2) Ahmed, M., Rahman, M. M., Islam, M. N., & Hossain, M. I. [8]. Development and quality evaluation of whey–fruit blended functional beverages: Physicochemical and sensory characteristics. Journal of Food Processing and Preservation, 47(2), e16589.
3) Ali, M. et al. [21]. Milk composition analysis using lactoscan technology. Dairy Science & Technology
4) APHA. [10]. Standard methods for the examination of water and wastewater. Washington DC.
5) AOAC. [18]. Official methods of analysis. Association of Official Analytical Chemists.
6) Ben-Hassan, R. M., & Ghaly, A. E. [45]. Continuous propagation of Kluyveromyces fragilis in cheese whey for pollution reduction. Applied Biochemistry and Biotechnology, 47(2–3), 89–105.
7) Bhavsagar, M. et al. [46]. Chhana whey beverage development. Indian Journal of Dairy Science.
8) Blois, M. [47]. Antioxidant determinations by the use of a stable free radical. Nature.
9) Central Statistical Agency of Ethiopia. [8]. Agricultural sample survey 2022/23 [48]. Addis Ababa, Ethiopia.
10) Claudia, M. et al. [49]. Polyphenol determination in food matrices. Food Chemistry.
11) De, S. [10]. Outlines of dairy technology. Oxford University Press.
12) Demirel, B., Yenigun, O., & Onay, T. T. [50]. Anaerobic treatment of dairy wastewaters: A review. Process Biochemistry, 40(8), 2583–2595.
13) El-Sohaimy, S. A., Abdelwahab, A. E., Brennan, C. S., & Aboul-Enein, A. M. [51]. Beetroot bioactive properties. Journal of Food Science and Technology, 57, 317–325.
14) FAO. [52]. FAOSTAT statistical database: Milk production data [53]. Food and Agriculture Organization of the United Nations.
15) García-Caparros, C. E., López-Rubio, A., & González-Martínez, C. [52]. Sustainable formulation strategies for whey-based beverages. Food Chemistry, 423, 137239.
16) Gilliland, S., & Speck, M. [54]. Antimicrobial activity of lactic acid bacteria. Applied Microbiology.
17) Gomez, K., & Gomez, A. [55]. Statistical procedures for agricultural research. Wiley.
18) ISO. [50]. Folin–Ciocalteu total phenol determination standard.
19) Jooyandeh, H. [56]. Whey beverage quality changes during storage. Food Science and Technology.
20) Kale, R. et al. [30]. Beetroot juice nutritional analysis. International Journal of Food Science.
21) Kotoupas, A., Georgala, A., & Koutinas, A. A. [57]. Whey utilization for ethanol production. Bioresource Technology, 98(3), 655–662.
22) Krivorotova, T., & Sereikaite, J. [23]. DNS method for reducing sugars. Food Chemistry.
23) Le, T. T., Nguyen, T. P., & Tran, T. T. [21]. Whey fermentation functional properties. LWT – Food Science and Technology, 157, 113097.
24) Liang, X., Jin, X., & Chen, Y. [58]. Beetroot bioactives and cardiovascular health. Trends in Food Science & Technology, 116, 281–298.
25) Malarmary, S. et al. [8]. Whey beverage optimization studies.
26) Mirzakulova, M., Tlegenova, Z., & Nurgozhin, T. [59]. Whey

valorization strategies for functional beverages. Foods, 14(6), 1123.
27) Ranganna, S. [60]. Handbook of analysis and quality control for fruit and vegetable products.
28) Russo, P., Capozzi, V., & Spano, G. [8]. Fermented functional dairy beverages. Trends in Food Science & Technology, 131, 130–144.
29) Sand, M., & Narayanan, S. [16]. Sensory evaluation in food science.
30) Sharma, R. et al. [61]. Storage quality of whey beverages.
31) Singh, P., Sharma, B., & Singh, G. [21]. Ginger bioactive compounds review. Food Chemistry Reviews, 1(3), 45–60.
32) Smither, R. [49]. Whey processing and utilization. International Journal of Dairy Technology, 61(2), 123–131.
33) Tanwar, B. et al. [21]. Dairy product compositional analysis. Food Science and Biotechnology.
34) Tavakoli, H. R., Jafari, S. M., & Ghorbani, M. [58]. Whey protein functional beverages. Food Chemistry, 364, 130360.
35) Vongsak, B. et al. [62]. DPPH antioxidant assay methodology. Natural Product Research.
36) Yeh, H. Y., Chuang, C. H., Chen, H. C., et al. [23]. Bioactive components analysis of ginger. LWT – Food Science and Technology, 55(1), 329–334.