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Storage stability evaluation of pasteurized whey based beverage

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Abstract
The study evaluated the storage stability of pasteurized whey-based beverage (WBB) by comparing it with a control (beverage without whey) over 140 days under refrigeration (5±1 °C). WBB was prepared as per the protocol developed by Navale (2023) in her dissertation and then pasteurized at 75±2 °C for 35 minutes. Sensory, physicochemical, functional, and microbial parameters were assessed at 20-day intervals. Sensory scores declined significantly after 120 days due to changes like sedimentation and loss of freshness. Physicochemical changes included decreased pH, protein, fat, and increased acidity, TSS, turbidity, and viscosity. Functional properties such as antioxidant activity, phenolics, and ascorbic acid decreased over time. Microbial growth increased, though coliforms remained absent. The study concluded that WBB remained acceptable up to 120 days, with moderate sensory acceptability.

Keywords: Raw mango pulp, changes, whey, Sweetner

Introduction
India, the world's largest milk producer, generates a significant quantity of whey a nutritious by-product rich in proteins, lactose, vitamins, and minerals mainly from paneer and chhana production. Traditionally underutilized, whey is now being recognized for its health benefits and functional properties, prompting its use in value-added beverages. Mango, particularly raw mango, is a popular fruit in India known for its rich nutrient profile and health benefits. However, around 75% of mangoes are lost pre-harvest due to natural drop, causing economic losses. To reduce whey wastage and utilize fallen raw mangoes, a whey-based beverage has been developed, offering a nutritious, sustainable alternative to synthetic beverages. While such innovations are promising, systematic studies on the shelf life and changes in sensory attributes, physicochemical parameters, functional properties and microbial safety of pasteurized whey-based beverage remain limited.

Materials and Methods
Materials and Equipment and Instrument
Whey was sourced from the Experimental Learning Unit, Division of AHDS, RCSM College of Agriculture, Kolhapur. Sweetner was procured from the Regional Sugarcane and Jaggery Research Station, Kolhapur. Raw green mangoes were sourced from the horticulture farm at RCSM College. Purified potable water was used in the preparation of control (non-whey) beverage. Essential utensils and instruments included a sieve, electronic weighing balance, hand blender, glassware, glass bottles (200 ml), crown caps, crown corking machine, and mixer-grinder. Analytical tools used were pH meter, cup viscometer, centrifugal separator, hot air oven, autoclave, nephelometer, laminar airflow, colony counter, spectrophotometer, color analyzer, and hand refract meter. Chemicals All chemicals used were of AR and GR grade, procured from Merck India Ltd. and Glaxo India Ltd.

Methodology

Clarification and Quality Evaluation of Whey

Whey sourced was tempered at 35 °C and clarified using a centrifugal separator at 5000 RPM to eliminate fat and suspended particles, The clarified whey was then analysed for its physicochemical properties.

Preparation of Raw Mango Pulp

Freshly harvested mangoes were washed to remove surface impurities and boiled for 30 minutes. After cooking, mangoes were peeled manually with minimal pulp loss. Pulp adhering to the seeds was scraped off and homogenized using a mixer grinder. The processed pulp was stored at-18 °C until use.

Preparation of Whey-Based Beverage (WWB)

WBB was prepared following the method developed by Navale (2023) [35]. The prepared beverage was filled into sterilized 200 ml glass bottles. Bottles were sealed with metal crown caps and pasteurized by immersion in water at 75±2 °C for 35 minutes. After cooling to room temperature, samples were stored under refrigeration (5±1 °C).

A control sample (beverage without whey) was prepared identically, replacing whey with water. Both samples were monitored over a six-month storage period for sensory, physicochemical, functional, and microbial changes. Two bottles per sample were tested every 20 days one for sensory/functional/physicochemical analysis, the other for microbial analysis. Evaluation continued until sensory quality declined to unacceptable levels.

Storage Study of Whey-Based Beverage

Two treatments were studied:

- **P0:** Beverage without whey (Control-BWW)
- **P1:** Whey-based beverage (WWB)

Both samples were stored at 5±1 °C and evaluated at 20-day intervals for sensory, physicochemical, microbial, and colour parameters. The study continued until the samples were deemed sensorially unacceptable, at which point analysis was discontinued.

Analytical methods

Sensory evaluation of whey based beverage and beverage without whey

For organoleptic evaluation of BWW and WWB judges familiar with the desirable attribute of beverage was selected. The both samples were evaluated for colour and appearance, flavour, taste, consistency and overall acceptability by using 9 point hedonic scale proposed by Amerine *et al*. (1965) [6].

Physico-chemical analysis of whey based beverage and beverage without whey

pH was determined as per method described in AOAC (2012) [3], acidity was determined as per AOAC (2005) [1], specific gravity was determined by specific gravity bottle as prescribed in BIS handbook (IS: SP 18; part XI, 1981 [18], total soluble solids was determined as per AOAC (2010) [2] by using hand refractometer, viscosity was measured by using brass made ford type efflux viscometer cup as suggested by Elena *et al*. (2020) [19], turbidity was determined as prescribed in IS: 3025 (part 10, 1984) [25], reducing sugar content was determined by Lane and Eynon method as described in AOAC (2012) [3], nonreducing sugar content was calculated by subtracting reducing sugar content from total sugar content which was determined by Lane-Eynon's method in AOAC (2012) [3], protein content was determined as per AOAC (2012) [3], fat content was determined using Gerber method as reported in FSSAI (2015) [20], Ash content was determined as per AOAC (2012) [3] by using muffle furnace and total solids content was estimated as described in AOAC (2012) [3].

Colour parameter analysis of whey based beverage and beverage without whey

Colour analysis was carried out using Color Flex (M/s Hunter Associates Laboratory, Inc., Reston VA, USA) a color measurement system equipped with a dual beam xenon flash lamp and universal software. The results are showed by the L*, a* and b* notations. It is a 3-D color representation method system where L* is indicating lightness of color and equals 0 for black and 100 for white. a* is showing the amount of red (0 to 60) or green (0 to-60) while b* is showing the amount of yellowness (0 to 60) or blueness (0 to-60).

Hue value

From L*, a* and b* hue value for BWW and WWB was calculated using formulae, prescribed in CIE standard Illuminate [ISO 1164-2:2007] 2006.

Where $h^0 = 90^0$ corresponding to yellow colour and $h^0 = 360^0$ to the violet colour.

$$\text{Hue } (h^0) = \text{arctangent } (b^*/a^*) \times 360/(2 \times 3.14)$$

Chroma value

From a* and b* chroma value for BWW and WWB was calculated using following formulae as prescribed in CIE standard Illuminate [ISO 1164-2:2007] 2006.

$$\text{Chroma } (C^*) = (a^{*2} + b^{*2})^{-2}$$

Colour index (E*)

From L*, a* and b* the colour index was computed according to the formulae prescribed in CIE standard Illuminate [ISO 1164-2:2007] 2006.

$$E^* = (L^{*2} + a^{*2} + b^{*2})^{-2}$$

Functional properties of whey based beverage and beverage without whey

Antioxidant activity was measured using the ferric reducing antioxidant power (FRAP) assay according to Benzie and Strain (1999) [11], total phenolic content was determined by using folin Ciocalteu method (kahkonen *et al*. 1999) [29], ascorbic acid was determined as suggested by Ranganna (1986) [40] and energy value was calculated by multiplying the protein (%) with 4, carbohydrate (%) with 4 and fat (%) with 9.1

Microbiological Analysis

BWW and WWB samples were analysed for the standard plate count (SPC), coliform count and yeast and mold count (YMC) by the methods as outlined in FSSAI manual (2015) [20] with slight modification.

Statistical analysis

The data obtained from the storage study of BWW and WWB were analysed using Factorial Completely Randomized Design (FCRD) for determining the overall mean, standard deviation, one way and two-way Analysis of Variance (ANOVA) and critical difference (CD) values were calculated. The statistical analysis was performed using Microsoft Excel (Microsoft office 2016) and OPSTAT software where treatments were two and replication were also two.

Results and discussion

Physico-chemical changes during the storage at 5^0C.

pH was higher in P0 (4.50±0.04) than P1 (4.29±0.04), with both showing a declining trend over 140 days due to lactic acid formation. Final pH values were 4.43±0.01 (P0) and 4.19±0.02 (P1). The decline was more pronounced in P1. Differences between treatments and storage periods were statistically significant (p<0.01). Similar trends were reported by Baljeet *et al*. (2013) [9], Sikder *et al*. (2001) [47], Ribeiro *et al*. (2023) [42], Zaman *et al*. (2023) [49], and Ahmed *et al*. (2023) [4]. Acidity increased during storage due to pH decline, from 0.36% to 0.42% (P0) and 0.44% to 0.51% (P1). Differences between treatments and storage periods were significant (p<0.01). Similar trends were observed by Ribeiro *et al*. (2023) [42], Zaman *et al*. (2023) [49], Ahmed *et al*. (2023) [4], and Harshal *et al*. (2023) [22]. Reducing sugar content increased significantly (p<0.01) during storage from 2.39% to 2.59% (P0) and 6.78% to 6.96% (P1) by day 140, likely due to sucrose inversion. Similar increases were reported by Divya & Archana Kumari (2009) [18], Ismail *et al*. (2011) [27], Ahmed *et al*. (2023) [4], and Bhat *et al*. (2014) [13]. Non-reducing sugar decreased during storage from 9.46% to 9.23% (P0) and 9.09% (P1) by day 140—likely due to sucrose hydrolysis under acidic conditions. The decrease was statistically significant (p<0.01). Similar trends were reported by Singh *et al*. (2014) [48], Divya & Archana Kumari (2009) [18], and Alane *et al*. (2017) [5]; Bhat *et al*. (2014) [13] observed a contrary increase.

Protein content in P0 and P1 decreased slightly during storage (P0: 0.04±0.01 to 0.265±0.011%; P1: 1.07±0.001 to 1.054±0.02%), but changes were statistically non-significant. Decline may be due to ascorbic acid-induced denaturation or protein breakdown. Similar trends reported by Jaspreet *et al*. (2015) [28], Divya & Archana Kumari (2009) [18], and others. Overall, protein content varied significantly across treatments and storage periods (p<0.01). Fat content in P0 and P1 decreased during storage (P0: 0.215±0.03% to 0.185±0.04%; P1: 0.325±0.04% to 0.305±0.01%), likely due to oxidation, enzymatic activity, or microbial degradation. Reduction was significant across treatments (p<0.01) but non-significant in P1. Similar trends reported by Bankar *et al*. (2021) [10], Zaman *et al*. (2023) [49], and Ahmed *et al*. (2023) [4]. Ash content, indicating mineral levels, decreased in P0 (0.42±0.007% to 0.28±0.07%) and P1 (1.06±0.007% to 0.93±0.07%) over 140 days, likely due to microbial use. Differences were significant (p<0.01). Similar declines reported by Mohamed *et al*. (2014) and Ahmed *et al*. (2023) [4], though Bankar *et al*. (2021) [10] observed an increase.

TS, representing all solids like proteins, fats, sugars, and minerals, decreased over 140 days from 12.91% to 12.54% (P0) and 18.69% to 18.33% (P1), likely due to reductions in protein, non-reducing sugar, fat, and ash. Changes were statistically significant (p<0.01), with P1 showing significance from 40 days onward. Similar declines were reported by Zaman *et al*. (2023) [49] and Mohamed *et al*. (2014), while some studies like Bankar *et al*. (2021) [10] noted increases. TSS, indicating sugars and soluble compounds, increased during storage from 11.25°Brix to 13.2°Brix (P0) and 15.05°Brix to 16.5°Brix (P1), showing significant differences (p<0.01). The rise, likely due to acid hydrolysis of complex sugars into monosaccharides, plateaued after 60 days. Similar increases were reported by Alane *et al*. (2017) [5] and Baljeet *et al*. (2013) [9] in whey-based fruit beverages. Viscosity, related to mouthfeel and macromolecular interactions, increased during storage from 5.63 to 6.03 cSt (P0) and 6.34 to 6.57 cSt (P1), with significant differences (p<0.01). The rise is likely due to solubilization of insoluble pulp components under acidic conditions and increased total solids. Similar trends were noted by Zaman *et al*. (2023) [49] and Alane *et al*. (2017) [5] in whey-based beverages. Turbidity, indicating cloudiness due to suspended particles, increased significantly during storage from 336 to 370.5 NTU (P0) and 362.5 to 401 NTU (P1) (p<0.01). The rise is attributed to colloidal instability, sedimentation, and aggregation of protein-fat complexes despite refrigeration. This trend aligns with findings in various fruit and whey-based beverages by Kumar *et al*. (2017) [32] and Oziyci *et al*. (2013) [37].

Table 1: Changes in Physico-chemical parameter of whey based beverage and beverage without whey during storage at 5^{0}C

Parameters	Storage periods							
	0	20	40	60	80	100	120	140
P_0								
pH	4.50±0.01	4.49±0.01	4.48±0.01	4.47±0.02	4.46±0.01	4.45±0.01	4.44±0.01	4.43±0.01
Acidity (%)	0.36±0.004	0.36±0.003	0.38±0.004	0.39±0.004	0.39±0.002	0.40±0.001	0.41±0.001	0.42±0.001
Reducing sugar (%)	2.39±0.01	2.44±0.01	2.46±0.01	2.48±0.01	2.51±0.01	2.53±0.02	2.56±0.02	2.59±0.01
Protein (%)	0.43±0.001	0.41±0.001	0.38±0.025	0.34±0.001	0.32±0.001	0.30±0.004	0.28±0.007	0.27±0.011
Ash (%)	0.42±0.007	0.40±0.007	0.38±0.007	0.36±0.007	0.34±0.007	0.32±0.007	0.29±0.007	0.28±0.007
TS (%)	12.91±0.01	12.89±0.01	12.80±0.03	12.73±0.01	12.69±0.01	12.61±0.01	12.55±0.02	12.54±0.02
TSS (^{0}Brix)	11.25±0.04	11.65±0.04	11.95±0.04	12.35±0.04	12.65±0.04	12.89±0.06	13.1±0.07	13.2±0.07
Viscosity (cSt)	5.63±0.007	5.81±0.007	5.84±0.007	5.87±0.011	5.91±0.011	5.95±0.011	6.00±0.007	6.02±0.011
Turbidity (NTU)	336±0.71	345.5±0.35	355.5±0.35	363±0.71	367.5±0.35	371±0.71	372.5±0.35	373.5±0.35
Specific gravity	1.042±0.0004	1.045±0.0004	1.048±0.0004	1.050±0.0004	1.052±0.0004	1.054±0.0004	1.056±0.0004	1.057±0.0004
P_1								
pH	4.29±0.01	4.27±0.01	4.26±0.01	4.23±0.01	4.22±0.01	4.21±0.01	4.20±0.01	4.19±0.02
Acidity (%)	0.44±0.001	0.45±0.002	0.46±0.001	0.47±0.000	0.48±0.001	0.49±0.001	0.50±0.001	0.51±0.001
Reducing sugar (%)	6.78±0.02	6.83±0.02	6.87±0.01	6.88±0.01	6.91±0.01	6.94±0.01	6.95±0.02	6.96±0.01
Protein(%)	1.072±0.001	1.07±0.001	1.07±0.001	1.06±0.001	1.06±0.001	1.06±0.001	1.05±0.001	1.05±0.002
Ash (%)	1.06±0.007	1.04±0.007	1.03±0.011	1.00±0.007	0.98±0.007	0.96±0.007	0.94±0.007	0.93±0.007
TS (%)	18.69±0.01	18.64±0.01	18.57±0.01	18.51±0.02	18.46±0.01	18.43±0.01	18.3 5±0.01	18.33±0.02
TSS (^{0}Brix)	15.05±0.04	15.3±0.07	15.6±0.07	15.85±0.04	16.05±0.04	16.25±0.04	16.45±0.04	16.55±0.04
Viscosity (cSt)	6.34±0.007	6.38±0.007	6.42±0.007	6.46±0.007	6.48±0.007	6.52±0.007	6.55±0.011	6.56±0.011
Turbidity (NTU)	362.5±0.35	368.5±0.35	371.5±0.35	374.5±0.35	377.5±0.35	380.5±0.35	390.5±0.35	401±0.71
Specific gravity	1.065±0.0004	1.069±0.0004	1.071±0.0004	1.073±0.0004	1.075±0.0004	1.077±0.0004	1.079±0.0004	1.080±0.0004

*Mean score±SE of two replications

Instrumental colour value changes during the storage at 5 °C

L* (lightness) increased significantly for P0 (19.58 to 30.63) and P1 (22.00 to 28.67) during storage (p<0.01), possibly due to protein aggregation (Giroux *et al*., 2008). Koffi *et al*. (2005) [31] and Ribeiro *et al*. (2023) [42] reported L* decreases in whey beverages, while Nedanovska *et al*. (2022) [36] observed L* increase in mango-flavored drinks. Rather *et al*. (2024) [41] noted a decrease in L* in soy whey pineapple beverage. a* (red/green tone) increased in P0 (3.50 to 5.20) and slightly decreased in P1 (5.53 to 5.28) over 140 days (p<0.05), likely due to sugar-amine interactions (Gao *et al*., 2024) [21]. Nedanovska *et al*. (2022) [36] saw a* rise then fall in mango drinks; Kumar *et al*. (2021) [31] reported a*-9.39 in whey beverages. Rather *et al*. (2024) [41] observed a* decrease in soy whey pineapple beverage. b* value (yellow-blue chroma) increased from 7.93 to 11.26 (P0) and 9.09 to 12.95 (P1) over 140 days. All changes were statistically significant (p<0.01). Similar increase reported by Nedanovska *et al*. (2023) [36] in mango beverages; Kumar *et al*. (2021) [31] noted a b* of 25.18 in fresh synbiotic whey beverages. Rather *et al*. (2024) [41] observed a decrease in soy whey drinks. Rise attributed to Maillard reaction (Gao *et al*., 2024) [21].

Hue angle decreased during 140 days from 66.26 to 64.48 (P0) and 68.75 to 67.81 (P1), with a peak at day 40 in P1. Changes were significant (p<0.01). Similar trends were observed by Koffi *et al*. (2005) [31] in whey banana beverage and Rizzola & Cortellino (2017) [16] in ricotta whey beverages. Decline attributed to Maillard browning (Berg & van Boekel, 1994; Pellegrino *et al*., 1995) [12, 39]. Chroma (colour intensity) increased from 8.70 to 12.44 (P0) and 9.76 to 13.99 (P1) over 140 days. Changes were statistically significant (p<0.01). Similar trends were observed by Koffi *et al*. (2005) [31] in UHT whey banana beverage and Rizzolo & Cortellino (2017) [43] in ricotta whey-based fruit beverages. Increase linked to rising a* and b* values (Koffi *et al*., 2005) [31]. Colour Index increased from 21.42 to 33.06 (P0) and 24.07 to 31.90 (P1) over 140 days. Differences were significant for periods and interactions (p<0.01). Similar colour index values were noted by Rizzolo & Cortellino (2017) [43] in ricotta whey beverages. A decline was reported by Baba *et al*. (2016) [8] in whey-based orange beverage. Changes attributed to pigment degradation, Maillard reaction, and colloidal interactions (Cortes *et al*., 2008; Sady *et al*., 2013) [16, 44].

Table 2: Changes in instrumental colour value parameter of whey based beverage and beverage without whey during storage at 5^{0}C

Parameters	Storage periods							
	0	20	40	60	80	100	120	140
P_0								
L*	19.58±0.01	21.07±0.02	22.63±0.08	25.66±0.07	27.18±0.11	28.85±0.07	29.91±0.05	30.63±0.15
a*	3.50±0.03	3.55±0.05	3.62±0.07	3.80±0.01	4.25±0.07	5.01±0.04	5.22±0.05	5.28±0.05
b*	7.97±0.007	8.01±0.01	8.23±0.02	8.79±0.02	9.28±0.02	10.29±0.01	10.78±0.03	11.26±0.02
Hue(^{0})	66.26±0.17	66.06±0.24	66.26±0.36	66.60±0.00	65.39±0.33	64.04±0.06	64.14±0.14	64.88±0.16
Chroma	8.70±0.02	8.76±0.03	8.99±0.05	9.58±0.06	10.20±0.00	11.44±0.04	11.97±0.05	12.44±0.04
P_1								
L*	22.00±0.04	22.63±0.08	23.1±0.11	23.85±0.07	24.86±0.10	26.11±0.06	27.59±0.12	28.67±0.13
a*	3.53±0.03	3.63±0.08	3.75±0.14	3.87±0.08	4.2±0.04	4.61±0.02	5.18±0.07	5.28±0.05
b*	9.09±0.01	9.51±0.02	10.32±0.03	11.03±0.04	11.69±0.04	12.42±0.02	12.78±0.04	12.95±0.02
Hue(^{0})	68.75±0.14	69.09±0.38	70.02±0.65	70.67±0.33	70.24±0.10	69.61±0.07	67.94±0.22	67.81±0.16
Chroma	9.76±0.01	10.18±0.05	10.98±0.08	11.69±0.06	12.42±0.05	13.24±0.03	13.79±0.06	13.99±0.04

*Mean score±SE of two replications

Functional properties changes during the storage at 5 °C

Antioxidant content decreased during storage from 68.48 to 58.34 mg/g AAE (P0) and 87.32 to 81.12 mg/g AAE (P1) over 140 days. Differences were statistically significant for treatments and periods (p<0.01), though P1 showed non-significant decline. Similar reductions were reported by Ribeiro *et al*. (2023) [42] and Rather *et al*. (2024) [41] in functional beverages. Decline attributed to phenolic degradation and oxidation during storage (Parra *et al*., 2016) [38], although lactic fermentation may also generate antioxidant compounds (Dahal *et al*., 2020) [17]. Total phenolic content declined during storage from 19.94 to 15.77 mg/g GAE (P0) and 20.7 to 17.79 mg/g GAE (P1) over 140 days. This decline was statistically significant (p<0.01) for both treatments and periods. Similar reductions were noted by Ribeiro *et al*. (2023) [42] and Sharma *et al*. (2019) [45]. While Naik *et al*. (2023) [34] observed an initial increase until day 21, a decrease followed by day 28. The reduction may be due to polymerization or binding of phenolics with proteins forming insoluble complexes (Kumar & Manimegalai, 2005; Sharma & Thakur, 2017) [32, 46].

Ascorbic acid content declined during 140 days of storage from 3.67 to 3.47 mg/100 ml (P0) and 3.67 to 3.40 mg/100 ml (P1). This reduction was statistically significant (p<0.01) for treatments and periods. The decline is likely due to auto-oxidation, light exposure, or degradation into by-products like furfural and hydroxymethyl furfural (Aruna *et al*., 1997; Sharma & Thakur, 2017) [7, 46]. Similar trends were observed by Ismail *et al*. (2011) [27], Baljeet *et al*. (2013) [9], and Ahmed *et al*. (2023) [4] in whey-based fruit beverages. Energy value represents the total calories derived from proteins, fats, and carbohydrates. A decreasing trend was observed over 140 days of storage, from 51.05±0.03 Kcal/ml to 49.98±0.07 Kcal/ml for P0 and from 72.18±0.01 Kcal/ml to 71.18±0.03 Kcal/ml for P1. The decline is attributed to reductions in macronutrients like fat, protein, and carbohydrates during storage. Statistically, the changes were significant for treatments and periods (p<0.01). Comparable results were reported by Kanchana *et al*. (2021) [30], noted energy values of whey-based herbal drinks ranging from 58.44 to 66.68 kcal/100g depending on the herbal additions.

Table 3: Changes in functional properties of whey based beverage and beverage without whey during storage at 5^0C

Properties	Storage periods							
	0	20	40	60	80	100	120	140
P_0								
Antioxidant activity (mg/g AAE)	68.48±0.72	67.97±1.25	67.02±0.69	65.52±0.72	63.52±0.48	60.35±0.64	59.28±0.51	58.34±0.71
Total Phenolic Content (mg/g GAE)	19.94±0.39	19.64±0.35	19.55±0.39	19.36±0.53	17.65±0.32	16.95±0.37	15.88±0.62	15.77±0.46
Ascorbic acid (mg/100 mL)	3.67±0.007	3.64±0.011	3.60±0.007	3.56±0.011	3.52±0.011	3.50±0.011	3.48±0.011	3.47±0.007
Energy value (Kcal/100 mL)	51.05±0.03	50.97±0.04	50.72±0.07	50.51±0.01	50.38±0.01	50.14±0.02	50.01±0.06	49.98±0.07
P_1								
Antioxidant activity (mg/g AAE)	87.32±0.69	85.94±0.49	85.26±0.52	84.47±0.37	83.75±0.42	83.01±0.37	81.85±0.85	81.20±1.10
Total Phenolic Content (mg/g GAE)	20.7±0.14	20.52±0.19	20.22±0.19	19.81±0.17	18.87±0.37	18.63±0.35	17.95±0.36	17.79±0.40
Ascorbic acid (mg/100 mL)	3.67±0.007	3.60±0.004	3.58±0.007	3.52±0.004	3.49±0.004	3.47±0.007	3.43±0.007	3.40±0.004
Energy value (Kcal/100 mL)	72.18±0.01	72.07±0.05	71.81±0.01	71.69±0.01	71.54±0.04	71.50±0.02	71.23±0.04	71.18±0.10

*Mean score±SE of two replications

Microbial changes during the storage at 5 °C

Total plate count (TPC) increased significantly during storage, rising from 0.45±0.02 × 10^4 cfu/ml to 5.10±0.07 × 10^4 cfu/ml in P0 and 0.55±0.04 × 10^4 cfu/ml to 6.40±0.07 × 10^4 cfu/ml in P1. Statistical analysis showed significant differences for treatments, periods, and their interaction (p<0.01). Similar increases were reported by Divya and Archana Kumari (2009) [18] in whey-based guava beverage, Alane *et al*. (2017) [5] in whey-based mango beverage, Ismail *et al*. (2011) [27], and Rather *et al*. (2024) [41] in soy whey pineapple beverage. Yeast and mold counts increased significantly over storage, rising from 0 to 0.81±0.01 × 10^2 cfu/ml in P0 and 0 to 1.00±0.01 × 10^2 cfu/ml in P1 by day 140. Statistical analysis showed significant differences for treatments, periods, and interactions (p<0.01). The increase is likely due to environmental contamination. Similar trends were reported by Alane *et al*. (2017) [5] in whey-based mango herbal beverage, Harshal *et al*. (2023) [22] in whey-based orange beverage, and Rather *et al*. (2024) [41], in soy whey fortified pineapple juice. No coliform bacteria were detected in either P0 or P1 samples throughout the entire 140-day storage period. This absence aligns with findings by Alane *et al*. (2017) [5] in whey-based mango herbal beverage, Hassan *et al*. (2015) [23] in fruit-flavored milk beverages, and Ismail *et al*. (2011) [27] in whey-based mango beverage.

Table 4: Changes in microbial analysis of whey based beverage and beverage without whey during storage at 5^0C

Microbial counts	Storage periods							
	0	20	40	60	80	100	120	140
P_0								
Standard plate count (10^4 cfu/mL)	0.45±0.04	0.65±0.04	0.96±0.02	1.30±0.04	2.30±0.07	3.58±0.05	4.25±0.18	5.10±0.07
Yeast and Mold count (10^2 cfu/mL)	0	0.25±0.01	0.34±0.02	0.43±0.01	0.51±0.01	0.64±0.01	0.74±0.01	0.81±0.01
Coliform count (cfu/mL)	ND	ND	ND	ND	ND	ND	ND	ND
P_1								
Standard plate count (10^4 cfu/mL)	0.55±0.04	0.88±0.06	1.28±0.02	2.90±0.07	4.35±0.11	5.65±0.11	5.95±0.04	6.40±0.07
Yeast and Mold count (10^2 cfu/mL)	0	0.23±0.02	0.38±0.01	0.47±0.01	0.54±0.01	0.65±0.01	0.79±0.01	1.00±0.01
Coliform count (cfu/mL)	ND	ND	ND	ND	ND	ND	ND	ND

*Mean score±SE of two replications

Changes in sensory qualities of beverage during storage at 5 °C

The colour and appearance, flavour, taste, consistency and overall acceptability score of both samples decreased significantly during the storage and this was more pronounced in beverage without whey. The colour and appearance score decreased with increase in storage which might be due to chemical reactions that led to the formation of brown pigment and hence made the product less acceptable. Similarly, findings were recorded by Divya and Archana kumari 2009, Baljeet *et al*. 2013 [18, 9] and Naik *et al*. 2023 [34]. There was decrease in flavour score which might due to loss of volatile aromatic substance, microbial activity and biochemical changes during storage. These are in conformity with Divya and Archana kumari 2009, Baljeet *et al*. 2013 [18, 9] and Sharma *et al*. 2020. The taste score also declined with advancement of storage which might be due to acid production and off flavour development with ascending of the storage Bankar *et al*. 2021 [10] and Rather *et al*. 2024 [41] also recorded same trend. The consistency declined with storage time it might be due to sedimentation and protein aggregation over the storage, analogous finding was recorded by Harshal *et al*. 2024 [22] and Rather *et al*. 2024 [41]. The overall acceptability also decreased as the time proceed are attributed as decline in colour and appearance, flavour, taste and consistency. Similar observations were reported by Divya and Archana Kumari 2009 [18], Baljeet *et al*. 2013 [9] and Ahmed *et al*. 2023 [4]. It was observed that though there was decrease in sensory attributes but yet it was acceptable till 120 days of storage study.

Conclusion

Pasteurized whey-based beverage demonstrated good storage stability, maintaining quality for up to 120 days under refrigerated conditions when packaged in sterilized glass bottles sealed with crown metal caps. Throughout the storage period, all sensory attributes gradually declined, yet the product remained acceptable up to the 120-day mark. Physicochemical analyses revealed a decrease in pH, proximate composition, total solids (TS%), while acidity, reducing sugars, total soluble solids (TSS), specific gravity, viscosity and turbidity, exhibited an increasing trend. Additionally, important functional parameters such as antioxidant activity, total phenolic content, ascorbic acid, and energy value showed a significant decline during storage. Microbial evaluation indicated an increase in standard plate count (SPC) and yeast and mold count (YMC) over time; however, coliform bacteria were absent throughout the entire 140-day storage period, reflecting effective processing methods and adherence to hygienic conditions.

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