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Standardisation and quality evaluation of whey incorporated mango RTS beverage

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Abstract

Whey, a protein-rich by-product of dairy processing, was incorporated into mango ready-to-serve (RTS) beverages to enhance their nutritional and functional properties. Beverages were formulated with varying proportions of whey (20-100%), while mango juice (50%), sugar (43.75 g), citric acid (1.25 g), and potassium metabisulphite (0.06 g) remained constant. Sensory evaluation using a nine-point hedonic scale identified the 70% whey and 30% mango juice formulation (T_5) as the most acceptable with respect to appearance, flavor, taste, texture, and overall acceptability. Physicochemical parameters including acidity, pH, TSS, total sugar, reducing sugar, non-reducing sugar, energy, protein, beta-carotene, vitamin C, total ash, calcium, iron, sodium, potassium, phosphorus was monitored over 30 days of refrigerated storage. Whey addition significantly increased protein and mineral levels, while minor declines in vitamin C and beta-carotene were noted due to oxidative degradation. Storage related changes, such as increased acidity, decreased pH, and sugar transformations, were consistent with biochemical interactions between whey and fruit components. The results demonstrate that whey enriched mango RTS beverages retain enhanced nutritional and sensory qualities during storage, indicating their potential as protein fortified, functional beverages for health-conscious consumers.

Keywords: Whey protein, Mango RTS beverage, functional beverage, physicochemical properties, sensory evaluation, storage stability

Introduction

The global food industry is quickly shifting toward functional beverages that go beyond basic nutrition. Unlike regular drinks that provide hydration and energy, these beverages are designed to give specific health benefits, such as boosting metabolism, strengthening immunity, and lowering the risk of chronic diseases. This change is driven by rising health awareness, urbanisation, and the increasing cases of obesity, diabetes, and heart problems. As a result, functional beverages have become one of the fastest-growing areas in the global food market, reflecting changing consumer preferences and the demand for healthier options.

Fruit-based ready-to-serve (RTS) beverages hold a significant place in the functional beverage sector because of their natural taste, freshness, and rich nutrient profile. Fruits provide essential vitamins, minerals, fibre, and phytochemicals that helps to reduce oxidative stress, regulate inflammation, and support overall health. Incorporating fruits into functional beverages not only enhances flavor and visual appeal but also boosts their nutritional value. Such products align with consumer demand for minimally processed, plant-based, and health-promoting alternatives to synthetic drinks.

Whey, a nutrient-rich by-product of cheese and paneer production, presents an increasing challenge for the modern dairy industry. Rather than being discarded, whey can be repurposed as a valuable functional food ingredient due to its rich composition of high-quality proteins, bioactive peptides, lactose, and essential minerals. Key proteins, including β-lactoglobulin and α-lactalbumin, are abundant in essential amino acids, particularly branched-chain amino acids (BCAAs), which play a crucial role in supporting muscle growth, tissue repair, and overall metabolic health.

Whey, a powerhouse of bioactive compounds, offers a multitude of health benefits. Whey-derived peptides demonstrate antioxidant, blood pressure-lowering, cholesterol-reducing, antidiabetic, and immune-enhancing effects. Regular consumption of whey is also associated with improved gut health, increased satiety, better weight management, and a lower risk of cardiovascular and metabolic disorders. These diverse properties position whey as a valuable ingredient for the development of functional beverages aimed at promoting preventive health.

Whey is nutritious, but its taste is bland and not very popular with consumers. Adding fruits can improve the flavor, color, and overall appeal of whey-based drinks. Fruits enhance the flavor, color, and palatability of whey-based drinks while also adding natural antioxidants, phytochemicals, and dietary fibre. This combination creates multifunctional beverages that meet modern expectations for taste, nutrition, and sustainability.

Materials and methods

Collection of raw materials

The cow's milk required for the preparation of whey water was procured from the dairy plant of Kerala Veterinary and

Animal Science University, Mannuthy, Thrissur. Mango, sugar and other raw materials were purchased from the local market.

Development of whey

Milk was heated at 84°C for 10 minutes, cooled to 72°C, and coagulated with a 1.5% citric acid solution. The paneer was separated by filtration, and the whey was boiled for 15 minutes to precipitate whey proteins, followed by filtration to obtain clear whey, which was then cooled at room temperature (Pandey *et al.*, 2019) [17].

Development of fruit juice

Ripe and mature mangoes are selected, washed thoroughly, and peeled. Then peeled fruits are cut into pieces and subjected to juice extraction. The extracted juice is strained to obtain clear mango juice (FSSAI, 2020) [6].

Table 1: Treatments for the standardisation of whey incorporated mango RTS beverages

Treatments	Whey (ml)	Mango juice(ml)	Water (ml)	Sugar (g)	Citric acid(g)	KMS (g)
T_1	-	50	404.94	43.75	1.25	0.06
T_2	404.94	50	-	43.75	1.25	0.06
T_3	364	50	40.94	43.75	1.25	0.06
T_4	324	50	80.9	43.75	1.25	0.06
T_5	283.4	50	121	43.75	1.25	0.06
T_6	243	50	162	43.75	1.25	0.06
T_7	202	50	202	43.75	1.25	0.06
T_8	162	50	243	43.75	1.25	0.06
T_9	121	50	283	43.75	1.25	0.06
T_{10}	81	50	324	43.75	1.25	0.06

Physico-chemical evaluation of whey incorporated mango RTS beverage

The physico-chemical properties like acidity, pH, TSS, total sugar, reducing sugar, non- reducing sugars, energy, protein, beta carotene, vitamin C, total ash, calcium, iron, sodium, potassium and phosphorus of the best selected whey incorporated RTS beverage were determined along with control.

Acidity

Acidity of RTS beverage samples was estimated by A.O.A.C. (2023) [2]. RTS beverage sample (10 g) was mixed thoroughly with 30ml of lukewarm distilled water. It was titrated against 0.1N NaOH using phenolphthalein as indicator.

$$\text{Acidity (\%)} = \frac{\text{Titre value} \times \text{Normality} \times 90 \times 100}{\text{Weight of sample} \times 1000}$$

pH

5g samples of RTS beverage were homogenised for 30 seconds in 100 ml of hot distilled water and vacuum filtered through Whatman filter paper. A 25ml aliquot was pipetted into a beaker and the pH was measured using a pH meter (A.O.A.C, 2023) [2].

TSS

Total Soluble Solids (TSS) of the RTS beverages were determined using a hand refractometer. The readings were taken at room temperature and expressed as degree brix (Ranganna, 2017).

Reducing sugars

25 ml of sample was transferred to a conical flask. It was then neutralised with 1N sodium hydroxide solution in the presence of phenolphthalein. Clarification of the neutralised mixture was done by the addition of 2 ml of lead acetate. The excess amount of lead acetate was removed by adding 2 ml of potassium oxalate. It was then allowed to stand for 10 minutes for the settlement of the precipitate. The solution was filtered through Whatman's No. 1 filter paper. It was then made upto 250 ml. Aliquot of the solution was titrated against a boiling mixture of fehlings solution A and B using methylene blue as indicator. End point of the reaction is the appearance of brick red colour (Ranganna, 2017). The reducing sugars present in the food mixture were computed using the formula as follows.

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's factor} \times \text{dilution} \times 100}{\text{Titre value} \times \text{weight of the sample}}$$

Total sugar

The total sugar was determined using the method suggested by Ranganna (2017). From the clarified solution used for the estimation of reducing sugar, 50 ml was taken. This solution was gently boiled after adding citric acid and water. The volume was made upto 250 ml after neutralising the solution with sodium hydroxide. The aliquot of this solution was titrated against Fehling's solution A and B. The total sugar content was expressed as percentage.

$$\text{Total sugars(\%)} = \frac{\text{Fehling's factor} \times 250 \times \text{dilution} \times 100}{\text{Titre value} \times 50 \times \text{weight of the sample}}$$

Non-reducing sugar

The quantity of reducing sugar was subtracted from that of total sugar multiplied by a conversion factor of 0.95 to get non-reducing sugar.

Non-reducing sugar (g) = Total sugar (g) - Reducing sugar (g) × 0.95

Energy

The energy content of whey incorporated RTS beverages was computed according to Gopalan *et al.* (1989) [7] and

expressed as kcal. The energy present in sample was calculated as per the formula given below.
Energy (Kcal) = (CHO × 4) + (Protein × 4) + (Fat × 9)

Protein

Protein was estimated by the method of A.O.A.C. (2023) [2]. A 0.2 g sample was digested with six ml of concentrated H, SO after adding 0.4 g of CuSO4 and 3.5 g K_2SO_4 in a digestion flask until the colour of the sample turned green. After digestion, it was diluted with water and 25 mL of 40% NaOH was added. The distillate was collected in 2% boric acid containing mixed indicators and then titrated with 0.2 N HCl to determine the nitrogen content. The nitrogen content thus estimated was multiplied by a factor of 6.25 to obtain the protein content.

β carotene

The sample (2 ml) was taken in a 100 ml glass stopper flask and added 10 ml of water saturated butanol (WSB). The contents of the flasks were mixed vigorously for 1 minute and kept undisturbed for 16-18 hours (overnight) at room temperature. Dark condition was maintained for the complete extraction of beta- carotene. The contents were again subjected to shaking and filtered completely through the Whatmann no. 1 filter paper into a 100 ml volumetric flask. The optical density (O.D) was measured at 440 nm (Sadasivam and Manickam, 1992) [23].

Vitamin C

The method outlines in the BIS Handbook (1989) was used to estimate the vitamin C concentration. Using TCA reagent or metaphophoric acid, a precisely weighed sample (approx. 5g) was pulverised in a mortar with acid-washed sand before being put into a 100ml graduated cylinder. The mixture was thoroughly agitated then either TCA reagent or metaphosphoric acid was added to bring the volume to 100ml. It was quickly filtered by using Whatman No. 1 filter paper. The final ascorbic acid concentration in the extract was maintained between 10 and 15g/ml. This filtrate was quickly titrated with the indophenol solution using 10ml of it. A blank titration was carried out using 11ml of the reagent and water, which was enough to make the mixture's volume equivalent to 15ml plus the volume of the indophenol solution needed for the direct titration.

Vitamin C content in the sample was calculated as follows

$$\text{Vitamin C content (mg/100g of the sample)} = \frac{(A \times B \times 1000)}{W}$$

Where,
A = Volume in ml of the indophenol solution used for titration,
B = Weight in mg of the ascorbic acid equivalent to 1ml of the indophenol solution,
W = Weight in g of the sample taken for the test

Total ash

Total ash was determined by the procedure of AOAC (1994) [1]. A clean and dry crucible was accurately weighted first and noted down. About 3 to 5 ml of the sample was placed in the crucible and again weighted so as to get the accurate weight of the sample. The crucible containing the sample was placed in an electric burner in a partially open manner for the sample to get charred with initial expulsion of smoke. After this, the crucible was placed in a muffle furnace and heated to 500 - 600^{o} C for 2 - 3 hours. Crucible was carefully removed from the furnace and cooled to room temperature and weighted again to get the reading.

$$\text{Ash content (\%)} = \frac{(Z - X) \times 100}{(Y - X)}$$

Where,
X - Weight of empty crucible in grams
Y - Weight of crucible + sample in grams
Z - Weight of crucible + ash in grams (after complete ashing)

Calcium

Calcium content of the selected fruit drinks were estimated by Atomic Absorption Spectrophotometric (AAS) method using the di acid extract prepared from the sample (Perkin-Elmer, 1982) [20]. A sample of 0.20 ml was predigested with 10 ml of 9:4 mixture of nitric acid and perchloric acid and make up the volume to 50 ml and used directly in Atomic Absorption Spectrophotometer for the estimation of calcium and expressed in mg 100 g $^{-1}$of sample.

Iron

Iron content present in selected fruit juice were determined using the method suggested by Perkin - Elmer (1982). 1 ml of the sample was pre - digested using 9:4 ratio of nitric acid and percholoric acid (10 ml). The prepared di acid extract of the fruit drink sample was used for estimation of iron in Atomic Absorption Spectrometer (AAS). Iron content present in the sample was expressed as mg 100 g $^{-1}$ of the sample.

Sodium

The sodium content of fruit juice was estimated using flame photometer as suggested by Jackson (1973) [9]. 1 ml of juice was digested in di acid and made up to 100 ml with distilled water. From this made up solution, 1 ml was directly fed to flame photometer and the reading was taken expressed the sodium content in mg per 100 ml of beverage.

Potassium

The potassium content present in the prepared fruit drink was estimated using the procedure suggested by Jackson (1973) [9]. The di acid extract of the fruit drink was directly read in the flame photometer and the potassium content was expressed in mg 100 g $^{-1}$ of sample.

Phosphorus

The method suggested by Jackson (1973) [9]. Phosphorus content was estimated by colorimetrically which gives yellow colour with nitric acid and vandate molybdate reagent. 1ml sample was pre-digested with 12 ml of 9:4 di acid and volume made up to 100 ml. The 5 ml of predigested aliquot, five ml of nitric acid, vandate molybdate reagent was added in to the volumetric flask and made up 50 ml with distilled water. After 10 minutes the optical density was red at 470nm. The phosphorus content was expressed in mg 100g $^{-1}$.

Stastistical analysis

The data was analysed using suitable statistical techniques. The best treatment was selected by applying Kendall's coefficient of concordance and nutritional parameters carried out paired sample t test.

Result and discussion

Standardisation and quality evaluation of RTS beverages

Standardisation of RTS beverage

RTS beverages were prepared following the standard procedure FSSAI (2020) [6]. In various combinations of whey and water were tested, with the whey percentage ranging from 100% to 20% and water varying between 10% to 80%, mango (50%), sugar (43.5), citric acid (1.25) and sodium benzoate (0.06) kept constant. Ready to serve beverage made with mango juice was taken as control (T$_1$).

Organoleptic evaluation of RTS beverage

A panel consisting of twenty judges was formed using a triangle test (Jellinek, 1985) [11]. The whey incorporated RTS beverage was evaluated organoleptically using a score card of nine-point hedonic scale. The selection of one highly acceptable blended RTS beverage combination from each set, along with a control (T$_1$), was selected for further studies. Mean scores obtained for the organoleptic evaluation of RTS beverages from whey and mango is presented in Table 2.

Table 2. Mean score of organoleptic evaluation of whey incorporated mango RTS beverage

Parameters	**Treatments**										
	T$_1$	**T$_2$**	**T$_3$**	**T$_4$**	**T$_5$**	**T$_6$**	**T$_7$**	**T$_8$**	**T$_9$**	**T$_{10}$**	**W**
Appearance	8.7 (8.00)	7.9 (3.10)	7.93 (3.31)	8.3 (5.05)	8.85 (8.86)	8.28 (5.31)	8.33 (5.55)	8.31 (5.55)	8.33 (5.76)	8.21 (4.52)	0.420**
Colour	8.71 (8.60)	7.8 (3.50)	7.75 (2.65)	8.1 (4.95)	9 (9.45)	8.18 (5.53)	8.08 (4.78)	8.06 (4.53)	8.18 (5.35)	8.23 (5.68)	0.526
Flavour	8.58 (8.76)	7.28 (2.33)	7.66 (4.14)	8 (5.74)	8.65 (8.81)	7.98 (5.88)	7.90 (5.21)	7.74 (4.74)	7.74 (4.69)	7.73 (4.69)	0.480**
Texture	8.47 (8.36)	7.50 (3.07)	7.69 (4.14)	7.95 (5.86)	8.69 (9.43)	7.85 (5.02)	7.96 (5.71)	7.73 (4.21)	7.77 (4.55)	7.76 (4.64)	0.488**
Taste	8.04 (6.67)	7.36 (3.31)	7.61 (4.83)	7.74 (5.60)	8.87 (9.86)	7.44 (3.21)	8.03 (6.88)	7.60 (4.26)	7.65 (4.45)	7.84 (5.93)	0.467**
Overall acceptability	8.26 (8.24)	7.63 (4.50)	7.53 (3.81)	7.76 (4.98)	8.69 (9.60)	7.69 (4.67)	7.74 (4.86)	7.65 (4.17)	7.79 (5.38)	7.67 (4.81)	0.426**
Total score	8.46	7.57	7.695	7.975	8.79	7.90	8.006	7.84	7.91	7.90	

Figures in parenthesis indicate mean rank scores, **significant at 1% level

The sensory characteristics of RTS beverages prepared with varying proportions of whey and water evaluated for appearance, colour, texture, taste, flavour, and overall acceptability (Table 2).

The appearance score was highest for T$_5$ (8.85), while T$_2$ received the lowest score of 7.9. The mean rank scores for appearance varied between 3.10 and 8.86 across the different treatments.

In terms of colour, T$_5$ received highest score (9), while T$_3$ received the lowest score of (7.75). The mean rank scores for colour ranged from 2.65 and 9.45 across the treatments.

The flavour of the RTS beverage was rated highest for T$_5$ (8.65), while T$_2$ had the lowest flavour score of (7.28). The mean rank scores for flavour ranged from 2.33 to 8.81. In terms of overall acceptability, T$_5$ again recorded the highest score (8.69), followed by T$_1$ (8.26), while the lowest score was observed in T$_3$ (7.53). The mean rank scores for overall acceptability ranged between 9.60 to 3.81.

The highest texture score was recorded by T$_5$ at 8.69, indicating a superior mouthfeel, while T$_8$ had the lowest texture score of 7.73. The mean rank scores for texture ranged from 4.21 to 9.43. Regarding taste, T$_{5\ \text{also}}$ achieved the highest score of 8.87, whereas T$_2$ had the lowest score of 7.36. The mean scores for taste varied between 3.31 to 9.86.

Based on the sensory evaluation, treatment T$_5$ (comprising 70% whey and 30% water) was identified as the most acceptable formulations for further quality assessment studies.

Selection of most acceptable RTS beverage

Quality evaluation of selected RTS beverage on storage

Based on the sensory evaluation, the RTS beverage formulation T$_5$ were chosen for further storage studies. The selected treatments from this set, along with the control, were evaluated for evaluated for physico-chemical and shelflife studies. The RTS beverage stored under refrigerated conditions had a shelf life of 45 days in preliminary trials. Hence, the quality evaluation of RTS beverage stored under refrigerated conditions, the evaluation was carried out for 30 days.

Physico-chemical composition of selected RTS beverage during storage

The physico-chemical constituents of the selected RTS beverages including acidity, pH, TSS, total sugar, reducing sugar, non-reducing sugar, protein, beta carotene, vitamin C, total ash, calcium, iron, sodium, potassium and phosphorus were estimated both initially and at the end of the storage period to assess changer over time.

Table 3. Physicochemical properties of whey-based mango RTS beverage

Parameters	Storage period					
	Initial Day			Final Day		
	Control	**T_5**	**t value**	**Control**	**T_5**	**t value**
Acidity (%)	0.39	0.30	3.36^{NS}	0.45	0.53	4.47^{NS}
pH	3.72	4.3	5.17^{NS}	3.6	3.4	3.46^{NS}
TSS(ºBrix)	13.18	15	6.17^{NS}	12.1	15.7	4.96^{NS}
Total sugar (%)	14.10	12.5	4.38^{NS}	13.9	11.7	7.67^{NS}
Reducing sugar (%)	4.70	4.01	6.82^{NS}	5.8	4.17	3.00^{NS}
Non - reducing (%)	7.6	7.49	2.32^{NS}	5.9	6.53	7.53^{NS}
Energy (Kcal)	53.8	65	5.89^{NS}	50.80	62	5.74^{NS}
Protein(gm)	0.46	2.4	6.95^{NS}	0.39	1.7	5.13^{NS}
Beta carotene(µg)	57	68	4.93^{NS}	50.2	57	4.37^{NS}
Vitamin C(mg)	21.70	15.06	4.59^{NS}	17.10	12.18	5.30^{NS}
Total ash (%)	0.21	0.35	1.64^{NS}	0.23	0.31	4.75^{NS}
Calcium (mg)	11.15	36.6	6.10^{NS}	10.13	31.9	7.60^{NS}
Iron(mg)	0.36	0.42	1.16^{NS}	0.24	0.23	1.73^{NS}
Sodium(mg)	3.5	17	6.71^{NS}	3.2	14	7.54^{NS}
Potassium (mg)	78.70	93	2.66^{NS}	73.65	86	2.48^{NS}
Phosphorus(mg)	5.58	26	7.47^{NS}	5.59	19	6.42^{NS}

*Significant at 5% per cent level, NS - non-significant, T_5 - 70% whey + 30% water

The whey-enriched beverage exhibited a lower initial titratable acidity of 0.30% compared to the control (0.39%). However, its acidity showed a notable rise over 45 days of refrigerated storage, attaining 0.53%, which surpassed the final titratable acidity of the control (0.45%). This progressive increase in acidity is generally attributed to the continued metabolic activity of residual lactic acid bacteria, which ferment remaining sugars and whey-derived lactose into organic acids, mainly lactic acid (Khedkar *et al.,* 2017) [12]. The elevated final acidity in the whey formulation reflects the impact of its compositional characteristics. This finding aligns with previous studies reporting that the higher residual lactose content in whey supports prolonged microbial metabolism and enhanced acid production, leading to greater accumulation of organic acids than in conventional beverages (He *et al.,* 2015) [8]. Thus, the persistent microbial activity confirms whey's role as a nutrient-dense substrate that promotes lactic acid bacteria growth. The pH of the whey-fortified beverage exhibited a marked decrease from 4.3 to 3.4 during 45 days of storage. This decline is mainly attributed to post-acidification, wherein the probiotic strain *Pediococcus acidilactici* BK01 continues to metabolize residual lactose and fermentable sugars into organic acids (Melia *et al.*, 2021) [14]. Although this acidification enhances microbial stability and suppresses pathogenic growth—thereby improving product safety—an excessive reduction in pH can adversely affect the beverage's quality. When the pH nears the isoelectric point of whey proteins (approximately 4.6, or slightly lower in heat-treated systems), conditions favor acid-induced protein denaturation and aggregation. At a pH of 3.4, these reactions lead to protein precipitation, observed as sedimentation, turbidity, or phase separation (Mousavi and Khodaiyan, 2022) [15, 16]. To prevent such undesirable changes, stabilizing hydrocolloids are commonly incorporated. These anionic polysaccharides maintain the dispersion of whey protein aggregates under low pH conditions (around 4.0) by imparting electrostatic and steric repulsion between protein molecules (Klemmer and Briesen, 2014) [13].

The whey-enriched beverage initially exhibited a lower Total Soluble Solids (TSS) value (13.18 °Brix) compared to the control (12.17 °Brix). However, over 45 days of storage, its TSS rose substantially to 15.7 °Brix, surpassing the control's final value of 12.17 °Brix. The reduction in TSS observed in the control sample reflects microbial utilization of available sugars, whereas the gradual increase in the whey-based beverage may result from the hydrolysis of macromolecules—particularly whey lactose—into smaller, more soluble components. This trend is consistent with the simultaneous rise in titratable acidity, as the accumulation of soluble organic acids produced during fermentation also contributes to elevated TSS levels (Wang and Fu, 2014) [27].

The whey-incorporated beverage initially contained a lower total sugar level (12.5%) compared to the control (14.1%). During 45 days of storage, both samples exhibited a notable decrease in sugar concentration, with the control showing a slight reduction to 13.9%, while the whey-based beverage declined more markedly to 11.7%. This reduction is primarily ascribed to the metabolic activity of lactic acid bacteria, which utilize available sugars—including whey lactose—to produce organic acids during storage (Khedkar *et al.,* 2017) [12]. These results align with the concurrent rise in titratable acidity and drop in pH, confirming that the conversion of sugars into acids plays a crucial role in the beverage's compositional changes (Khedkar *et al.,* 2017) [12]. The greater sugar depletion observed in the whey formulation underscores the need for optimising product formulation to preserve sweetness and sensory quality throughout its shelf life.

A significant increase in reducing sugar content was observed in both formulations during the 45-day storage period. In the control sample, levels rose from 4.70% to 5.8%, while in the whey-fortified beverage, they increased from 4.01% to 4.17%. This rise can be attributed to the hydrolytic breakdown of non-reducing sugars such as sucrose and lactose into simpler reducing sugars, including glucose, fructose, and galactose (Sakhale *et al.*, 2012) [24, 25]. The trend corresponds with the reduction in total sugar content, suggesting that although sugars are metabolized into organic acids, the simultaneous degradation of complex carbohydrates contributes to the accumulation of reducing sugars. These compositional alterations in the whey-based formulation may affect both sweetness perception and physicochemical stability during storage.

A marked reduction in non-reducing sugar content was observed in both beverages throughout the 45-day storage period. In the control, levels decreased from 7.6% to 5.9%, whereas in the whey-based formulation they declined from 7.49% to 6.53%. This decrease is primarily attributed to the hydrolytic conversion of complex non-reducing sugars, such as sucrose and lactose, into simpler reducing sugars like glucose and fructose (Sakhale *et al.,* 2012) [24, 25]. The observed decline aligns with the concurrent increase in reducing sugar levels and the overall decrease in total sugar content, indicating that carbohydrate hydrolysis is a key factor governing storage-related transformations. These compositional changes play a crucial role in determining the beverage's physicochemical properties and flavor profile (Sakhale *et al.*, 2012) [24, 25].

A noticeable reduction in energy content was observed in the whey-based beverage, which declined to 62 kcal after 45 days of storage, yet remained higher than that of the control (50.80 kcal). This decrease is mainly attributed to the fermentation-driven utilisation of carbohydrates, during which sugars are converted into organic acids and other metabolites with lower caloric value. Similar reductions in the energy content of whey-based beverages during storage have been reported by De Ancos *et al.* (2014) [5], supporting the role of microbial sugar metabolism as the primary contributing factor. Despite this decline, the whey-fortified beverage maintained a relatively higher energy density than the control, highlighting its potential as a functional formulation with sustained nutritional advantages. A significant reduction in protein content was recorded in the whey-fortified beverage, decreasing from 2.4% at the start of storage to 1.7% after 45 days. Conversely, the control showed only a minor decline from 0.46% to 0.39%, which was not statistically significant. The pronounced decrease in the whey-based formulation can be attributed to the sensitivity of whey proteins to acidic environments, resulting in denaturation, aggregation, and subsequent precipitation during storage. This observation aligns with previous findings on the behavior of whey proteins near their isoelectric point, where reduced pH promotes protein aggregation (Klemmer and Briesen, 2014) [13]. Despite the decline, the whey-incorporated beverage maintained a relatively higher protein content than the control, emphasising its value as a functional, protein-enriched formulation. A significant decline in beta-carotene content was observed in the beverage samples during storage. In the control, levels decreased from 57 to 50.2 µg/100 mL, whereas the whey-fortified beverage showed a more pronounced reduction from 68 to 57 µg/100 mL. This loss is primarily due to the susceptibility of beta-carotene to oxidative degradation when exposed to light, oxygen, and temperature variations during storage (Rodriguez-Amaya, 2018) [22]. Despite this decrease, the whey-based beverage maintained a higher beta-carotene concentration than the control, underscoring its potential as a nutritionally enriched functional product.

A notable difference in total ash content was observed between the two beverage formulations. The whey-fortified beverage initially showed a higher ash content (0.35%) compared to the control (0.21%). After 45 days of storage, this trend persisted, with values of 0.31% for the whey-based beverage and 0.23% for the control. The higher ash content in the whey formulation reflects the naturally rich mineral composition of whey protein concentrate (Jelen, 2003) [10]. Although the changes in ash content within each sample during storage were not statistically significant, the consistently greater mineral content of the whey-fortified beverage highlights its nutritional advantage and reinforces its potential as a functional food product.

A significant decline in Vitamin C content was observed in the beverage formulations over the 45-day storage period. In the control, levels decreased from 21.70 to 17.10 mg/100 mL, while in the whey-fortified beverage, Vitamin C dropped from 15.06 to 12.18 mg/100 mL. This reduction is primarily due to the high susceptibility of ascorbic acid to oxidative degradation when exposed to light, oxygen, and heat (De Ancos *et al.,* 2014) [5]. The control consistently retained higher Vitamin C levels than the whey-based formulation, possibly due to the destabilising effect of whey proteins or the lower initial concentration of the vitamin in the whey beverage. These results highlight the challenges of maintaining labile micronutrients in complex functional beverage systems.

A significant decrease in calcium content was observed in the beverages during storage. In the control, levels declined from 11.15 to 10.13 mg/100 mL, while the whey-fortified beverage experienced a larger reduction from 36.6 to 31.9 mg/100 mL. Despite this decline, the whey-based formulation consistently retained higher calcium concentrations than the control. The elevated calcium content in the whey beverage reflects the naturally high mineral content of whey protein, which provides bioavailable calcium. The moderate reduction over time is likely due to calcium phosphate precipitation under acidic conditions, affecting its solubility and measurable concentration (He *et al.*, 2015) [8]. These findings emphasise the nutritional advantage of the whey-fortified beverage.

A notable decrease in iron content was observed in the beverages over the storage period. In the control, levels declined from 0.36 to 0.24 mg/100 mL, while the whey-fortified beverage showed a reduction from 0.42 to 0.23 mg/100 mL. This decrease can be attributed to the pro-oxidant nature of iron, which may catalyse the degradation of other components such as Vitamin C and beta-carotene, as well as its susceptibility to acidic and oxygen-rich conditions during storage (Coupland, 2017) [4]. Although the whey beverage initially contained slightly higher iron levels, this advantage was not sustained, highlighting the challenges of maintaining the stability of transition metals in fortified beverage systems.

The sodium content of the control beverage showed a slight decrease from 3.5 mg/100 mL to 3.2 mg/100 mL after 45 days (t = 1.162), representing a non-significant change, whereas the whey-fortified sample (70% whey + 30% pineapple juice) experienced a significant reduction from 17 mg/100 mL to 14 mg/100 mL (t = 2.455). Higher overall t-values (6.714 and 7.543) confirmed significant differences in sodium levels between the two formulations. The decline during storage is likely due to protein-mineral interactions, precipitation, and pH-induced alterations that reduce measurable sodium (Patel, 2015) [18]. Despite this reduction, the whey-incorporated beverage retained considerably higher sodium content than the control, enhancing its overall mineral profile.

A notable difference in potassium content was observed between the two beverage formulations. The whey-fortified beverage initially contained 93 mg/100 mL, significantly higher than the control (78.70 mg/100 mL). After 45 days of storage, this difference remained significant, with the whey-pineapple formulation at 86 mg/100 mL and the control at 73.65 mg/100 mL. The elevated potassium content in the whey-based beverage reflects the naturally high potassium levels in whey protein concentrate (Jelen, 2003) [10]. Although both beverages experienced a slight, statistically non-significant decline over the storage period, potassium levels remained relatively stable. These findings demonstrate that whey incorporation substantially improves the mineral profile, highlighting its potential as a nutritionally enhanced functional beverage.

A significant difference in phosphorus content was observed between the two beverage formulations. The control beverage remained essentially unchanged during storage, with a minimal shift from 5.58 mg/100 mL to 5.59 mg/100 mL by day 45 (t = 2.311). In contrast, the whey-fortified beverage showed a notable decline from 26 mg/100 mL to 19 mg/100 mL (t = 2.897). Higher overall t-values between the formulations (7.479 and 6.429) confirmed significant differences in phosphorus levels. The reduction in the whey-based beverage is likely due to protein-mineral interactions and precipitation of protein-phosphate complexes under acidic conditions, which decrease soluble phosphorus (Patel, 2015; Singh and Gallier, 2017) [18, 19, 26]. Despite this decline, the whey-fortified beverage consistently maintained substantially higher phosphorus levels than the control, emphasising its superior mineral profile.

Discussion

Whey protein was incorporated into a fruit-based beverage to create a value-added product with enhanced nutritional and functional attributes. The formulation containing 70% whey and 30% mango juice showed the most favorable results, with significantly higher amounts of protein, ash, calcium, sodium, and potassium compared to the control, reflecting the rich mineral composition of whey. Despite modest reductions in Vitamin C and beta-carotene during storage due to oxidative effects, the whey-fortified beverage consistently maintained superior nutritional quality. The integration of whey not only boosted the protein and mineral content but also complemented the natural nutrients of the mango juice, resulting in a balanced combination of dairy and fruit-derived benefits. This innovative formulation meets the increasing consumer demand for functional, health-focused beverages and supports diversification within the functional beverage industry.

Conclusion

The study demonstrated that whey protein can be successfully incorporated into a fruit-based beverage to produce a value-added, nutritionally enhanced product with desirable functional properties. Among the tested formulations, the whey-fortified beverage (70% whey + 30% mango juice) proved the most promising, showing significantly higher levels of protein, ash, calcium, sodium, and potassium compared to the control, reflecting the mineral-rich nature of whey. Although some nutrients, such as Vitamin C and beta-carotene, experienced modest declines during storage due to oxidative degradation, the whey-based beverage consistently maintained superior nutritional quality. The inclusion of whey not only enriched the beverage with high-quality proteins and essential minerals but also complemented the natural nutrient profile of the fruit juice. This hybrid formulation offers a balanced matrix of dairy- and fruit-derived nutrients, catering to the growing consumer demand for functional, health-oriented beverages and supporting product innovation and diversification in the functional beverage industry.

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