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Research Article

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Effect of Storage Temperature and Interval on Sensory, Physico-chemical and Microbiological Properties of Orange-based Paneer Whey Beverage

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Abstract

With the potential to be the fastest growing functional beverage market, the aim of this study was to minimize the waste of oranges and whey from mass production and use these nutrients in the formulation of orange whey-based functional beverages. Whey contains 7.8-12% of total milk solids, 1.4-2.9% protein, 1.2-3.6% fat, 0.6-0.7% minerals and nearly all of the water-soluble vitamins found in milk. Paneer whey drinks were therefore prepared by blending different concentrations of 10, 15, 20, 25 and 30 % orange juice with 90, 85, 80, 75 and 70% whey and 8% sugar. Based on the statistical analysis of the sensory evaluation of the beverages, the treatment (T_4) contained 25:75 ratio of orange juice to whey and all beverages studied to examine the potency of orange and its preservation quality was judged to be the best among 0, 3, 6, 9, 12, and 15 at room temperature (28 + 2 °C) and refrigerated temperature (7 + 2 °C).

Keywords

Whey, Orange juice, Beverage, Functional

Introduction

Whey is the largest and most important by-product of the dairy industry. It is obtained during the manufacture of casein, cheese, paneer, channa and shrikhand. Whey protein is composed of 4 major protein fractions and 6 minor protein fractions. Major protein fractions include beta-lactoglobulin (65%), alpha-lactalbumin (25%), bovine serum albumin (8%) and immunoglobulins (2%) [1, 2]. Current global whey production is estimated at about 165 million tons, of which about 95% is cheese whey. In the absence of systematic studies and statistics, the projected value of whey production in India is estimated at 5 million tons per year. Whey contains total milk solids, protein, fat, minerals and water-soluble vitamins found in milk. Along with this whey also have high biological oxygen demand of 39,000-48,000 ppm [3] and the chemical oxygen demand of 60,000-80,000 mg/l [4], due to strict environmental regulations. Whey contamination is a big problem. The use of whey in the production of beverages, soft drinks and wine is therefore one solution to minimize the severity of the pollution problem [5].

Orange (*Citrus reticulata*) is important sub-tropical fruit originated in China and mostly popular in India. Oranges constitute a significant source of antioxidants (mainly vitamin C), polyphenol compounds (hydroxyl cinnamic acid and flavanones), phyto-chemicals (hesperidins and narigenin) and various vitamins and minerals. These components exhibit therapeutic properties such as anti-inflammatory, antihypertensive, diuretic, analgesic and hypolipidemic activities [6].

Orange and whey beverage could be an interesting and nutritious product in the developing market for functional foods. The health and nutritional benefits of orange imparts the value to the formulated beverage. This beverage is popular and cheaply available, acidic flavor of whey is also compatible with orange fruit juice and have many health beneficial with respect to polyphenolic content, antioxidant activity and protection against different vitamin deficiency disease, etc. Whey's nutritional benefits can be used with fruit juices, pulps, or concentrates in the development of value-added beverages. This is the most logical and economical way to use whey nutrients in the human food chain [7].

Many attempts have been made on utilization of whey in the formulation of various dairy products but, still there is a lot of scope to explore the possibilities for its utilization in beverage industries. In the present investigation we studied the process standardization, chemical composition, storage stability and sensory evaluation of paneer whey beverage using orange juice.

Materials and Method

Procurement of raw material

Paneer whey

Paneer whey samples were prepared by the method given by Aneja [8], and whey was separated from coagulum by filtration using a clean muslin cloth. The whey was held for another 2 min at 85 °C before being drawn into a container for further processing.

Orange juice

Fresh, well-ripened orange fruits (Nagpur Mandarin) were purchased from a local market and washed with clean water. The peel was removed, washed, ground with an electric mixer, and the uniform mass was filtered through a muslin cloth to obtain orange juice.

Packaging material

The orange-based paneer whey drink was packaged in glass bottles and stored at room and refrigerated temperatures during storage for further evaluation.

Preparation of orange-based paneer whey beverage

Five different types of whey-based orange beverages were prepared by blending various mixtures of whey and orange juice. A sugar content of 8% was used as a constant for each mixture (Table 1). The proportions of orange juice blended with paneer whey were 10%, 15%, 20%, 25% and 30% [9]. Products were evaluated based on their physiochemical, sensory, and microbiological profiles on days 0, 3, 6, 9, 12, and 15 at room temperature (28 + 2 °C) and refrigerated temperature (7 + 2 °C). Manufacturing process flow diagram of whey-based orange juice beverage.

Table 1: Treatment details of orange based paneer whey beverage.

Treatments	Orange juice (%)	Whey (%)	Sugar (%)
T_1	90	10	8
T_2	85	15	8
T_3	80	20	8
T_4	75	25	8
T_5	70	30	8

Physico-chemical analysis

Estimation of fat

The fat content of orange-based paneer whey beverage was determined using the Gerber method of fat estimation, following the guidelines of Methods of test for dairy industry [10]. Firstly, 10 ml of sulphuric acid was carefully measured and poured into a butyrometer using a tilt measure. Then, 10.75 ml of sample was extracted using a pipette and slowly transferred into the butyrometer. To this, 1.0 ml of amyl alcohol was added using an automatic tilt measure. The butyrometer was then securely sealed with a lock stopper, using a guiding pin, and the contents were thoroughly mixed. Subsequently, the butyrometer was placed in a water bath set at 65 °C for 5 min. Afterward, the butyrometer was placed in a centrifugal machine that rotated at 1100 rpm for 5 min. Once the process was completed, the butyrometer was carefully removed in an upright position, with the stopper end facing downwards. The fat reading was directly taken from the bottom of the fat column to the lower border of the meniscus on the scale of the butyrometer stem.

Estimation of protein

The protein content was determined as per Semi-Micro Kjedahls method of Manfee and Overman described in Official Methods of Analysis of the Association of Official Agricultural Chemists [11]. To begin the process, a 2 ml whey sample was collected and placed into a Kjeldahl's flask. Next, 25 ml of pure sulfuric acid, free of nitrogen, was added to the flask. An 11.0 g digestion mixture, consisting of 10.0 g of potassium sulphate and 1.0 g of copper sulphate solid, was then introduced to the flask. The flask's contents were heated until they became colorless, a process that lasted for an hour or more. Subsequently, the flask's contents were cooled and diluted with 100 ml of distilled water. The contents from the digestion flask were transferred to the distillation apparatus. To make the solution alkaline, 80 ml of a 50% NaOH solution was added, and litmus paper was used to confirm the alkalinity. To prevent any splashing during distillation, a small amount of pumice stone powder was added. In a separate beaker, 50 ml of a 0.1 N H_2SO_4 solution was prepared and 3 drops of methyl red indicator were added. This beaker was positioned below the condenser tube in a way that the tube dipped into the solution. The distillation process was stopped once about 200 ml of distillate had been collected in the beaker. The apparatus was disconnected, and the collected distillate was titrated against a 0.1 N NaOH solution until the methyl red indicator changed from red to yellow, indicating a neutral endpoint.

$$\%\ Nitrogen\ in\ sample = \frac{No.\ of\ ml\ of\ 0.1\ N\ NaOH\ required \times 0.0014}{Weight\ of\ sample} \times 100$$

The protein percentage was calculated by multiplying nitrogen percentage with factor 6.38.

$$\%\ Protein = \%\ Nitrogen\ in\ sample \times 6.38$$

Estimation of total sugars

The sugar content of paneer whey drink with orange juice was measured using the Lane and Eyon's method proposed by Ranganna [12]. Twenty-five ml of sample was weighted accurately in 200 ml conical flask and was diluted with 100 ml luke-warm water (35-45 °C) and neutralized it with 1 N NaOH. The content of the flask was mixed thoroughly. For the precipitation of protein in the sample, neutral lead acetate and potassium oxalate solution was used as precipitant. After precipitation, the content was filtered through filter paper into a 200 ml graduated flask. The precipitate and the paper were washed thoroughly with hot water and the washings were collected in the flask. The flask and contents were cooled and the volume was made up to the mark and filtrate was used for estimation of total sugar.

Determination of total sugar (as invert sugar): Filtrate obtained as per the method described above was hydrolyzed by boiling 25ml of filtrate with 5ml of 54% HCI for 10 min. After cooling the contents were neutralized with 1 N NaOH solution. The volume was titrated against 5 ml of each of Fehling's solution A and B and percent total sugar was determined as under:

$$\% \text{ Total sugar} = \frac{\text{Factor for Fehling's solution as invert sugar x 4}}{\text{Titer as invert sugar}}$$

Percent of total sugar was determined as under

$$\% \text{ Total sugar} = (\% \text{ Reducing sugar} + \% \text{ sucrose})$$

Estimation of acidity

Acidity of the whey beverage was determined as per the procedure described in Indian Standards Institution and Book for Food Analysis [13]. 10 ml of whey beverage sample was taken in porcelain dish, it was mixed with 10 ml of distilled water. Then two drops of phenolphthalein indicator was added and titrated against 0.1 NaOH solution.

$$\% \text{ acidity} = \frac{\text{Number of ml. of N/10 NaOH x 0.009}}{\text{Weight of sample } (\text{sp. gravity x volume})} \text{ x } 100$$

Estimation of pH

The pH was estimated as per the procedure described in Ranganna [12]. The pH of sample was measured by using digital pH meter at a temperature of 25 °C. At first the pH meter was standardized by using standard buffer solution of pH 4 and pH 9. The electrode of pH meter was directly dipped into the beverage sample and on the screen of pH meter, the pH was observed, which was recorded.

Sensory evaluation of whey drink

Whey beverage samples were evaluated for their acceptability in terms of sensory evaluation. Sensory evaluation was performed by presenting approximately 50 ml samples of the whey beverage to seven semi-trained panelists drawn from the staff of the Post Graduate Institute of Animal and Dairy Sciences. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. A 9-point hedonic scale was used to rate the samples in terms of sensory attributes such as taste, color and appearance, consistency, and overall acceptability. The 9-level pleasure scale contains a variety of rating scales. viz. Likes extremely (9), likes very much (8), likes moderately (7), likes slightly (6), neither likes nor dislikes (5), disliked slightly (4), dislikes somewhat (3), very dislike (2), very dislike (1) [14].

Microbiological quality

Microbiological qualities in respect of yeast and mould and standard plate count were determined by using potato dextrose agar and nutrient agar medium, respectively. The poured petri dishes were incubated at 25 °C temperature for 4 to 5 days and the develop colonies of said micro-organisms were observed and recorded as standards plate count and yeast and mould count per ml of paneer whey beverage sample [15].

Statistical analysis

Data were analyzed statistically by randomized block design and factor randomized block design as per the procedure given by Panse and Sukhatme [16]. Means between the periods of storage and conditions were compared by two-way analysis of variance (ANOVA) and the critical difference test as per Snedecor and Cochran [17].

Results

Sensory parameters

Flavour

A significant effect on the flavour scores of paneer whey beverage (T_4) in response of different temperature conditions and storage interval viz. 8.23, 7.22, 6.02, 5.67, 5.19 and 4.72 were seen from the paneer whey beverage stored at room temperature and obtained less score as compared to beverage stored at refrigeration temperature (Table 2). The initial flavour score 8.23 for flavour in the freshly prepared beverage decreased to 4.72 during 15 days of storage. The quality of paneer whey beverage was acceptable up to 9th days. Thus, the flavour scores of paneer whey beverage (T_4) showed that, the temperature conditions and storage intervals interaction was statistically significant ($p < 0.05$) and maximum storability was observed in refrigeration storage as compare with the ambient storage. The differences between the periods of storage and conditions were compared by two-way ANOVA and the critical difference test as per Snedecor and Cochran.

Colour and appearance

Beverage colour and appearance were affected by storage temperature and storage interval and decreased from an initial value of 8.27 to 4.41 and 5.10, respectively, by 15 days of storage (Table 2). Individual average ratings of color and appearance of paneer whey beverages prepared from a mixture of 75% paneer whey and 25% treated (T_4) orange juice at room temperature (28 + 2 °C) and refrigerated temperature (5 + 2 °C) were 5.96 and 6.80, respectively. The comparisons between storage times and conditions were compared by two-way ANOVA and Snedecor and Cochran critical difference tests. The temperature conditions and storage intervals interaction had shown significant ($p < 0.05$) effect on the colour and appearance score of orange-based paneer whey beverage.

Consistency

Individual average consistencies of paneer whey beverages made from a blend of 75% paneer whey and 25% processed

Table 2: Effect of room and refrigerated conditions on physico-chemical characteristics of paneer whey-based orange juice beverage (T_4) (mean + SE).

Treatments	0 - day	3 – day	6 - day	9 - day	12 - day	15 - day
Fat						
Room temperature	0.42	0.4	0.38	0.35	0.32	0.3
Refrigeration temperature	0.42	0.4	0.38	0.36	0.34	0.32
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.009		0.027	
Protein						
Room temperature	0.59	0.57	0.55	0.52	0.5	0.49
Refrigeration temperature	0.59	0.58	0.57	0.55	0.53	0.51
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.004		0.012	
Total sugar						
Room temperature	12.95	12.65	12.36	12.1	11.87	11.65
Refrigeration temperature	12.95	12.76	12.61	12.45	12.03	11.82
Interaction (A x B)	F -Test		SE ± (d)		CD at 5%	
	Sig.		0.05		0.15	
Acidity						
Room temperature	0.31	0.36	0.37	0.39	0.41	0.42
Refrigeration temperature	0.31	0.32	0.33	0.34	0.35	0.37
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.003		0.01	
pH						
Room temperature	4.25	4.18	4.05	3.82	3.43	3.36
Refrigeration temperature	4.25	4.22	4.16	4.13	3.58	3.54
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.06		0.13	

Note: SE ± (d)- Standard Error of Difference; CD–Critical Difference; A × B-Durations × Temperature condition; Sig.–Significant; Data are pooled mean of three replications. Significant ($p < 0.05$) variation between duration of the product and temperature conditions were assessed by F-Test.

orange juice (T_4) at room temperature (28 + 2 °C) and refrigerated temperature (7 + 2 °C) the values were 5.91 and 6.70, respectively (Table 2). The mean with different temperature conditions and interval significantly differs at the $p<0.05$ level. The temperature x interval interaction was statistically significant ($p < 0.05$). Differences between storage times and conditions were compared by two-way ANOVA and Snedecor and Cochran critical difference tests.

Overall acceptability

Effect of storage temperature and intervals on overall acceptability for treatment T_4 (25%) orange juice and 75% ranged from 8.25 to 4.35 and 5.01, respectively (Table 2). The data presented in table 2 shows that the reduction in overall acceptability scores might have been observed due to the cumulative effect of changes in flavour, consistency, colour, and appearance sensory scores of the orange-based paneer whey beverage during storage. The differences between the periods of storage and conditions were compared by two-way ANOVA and the critical difference test as per Snedecor and Cochran. The interaction of storage temperature x interval was statistically significant ($p < 0.05$).

Physico-chemical parameters

Fat content

The fat content of paneer whey blended with orange juice was recorded to be lower with an increased level of orange juice. It may be due to the lower amount of fat content in orange juice, viz. 0.18%. The mean fat content of whey beverage was 0.42, 0.40, 0.38, 0.35, 0.32, 0.30 and 0.42, 0.40, 0.38, 0.36, 0.34 and 0.32 on 0, 3, 6, 9, 12, and 15 days at room temperature and refrigerated temperature, respectively (Figure 1). The fat content also decreased significantly ($p \leq 0.05$) at room temperature and refrigeration temperature, respectively during storage. The differences between the periods of storage and conditions were compared by two-way ANOVA and the critical difference test as per Snedecor and Cochran.

Protein content

Effect of storage on the protein of beverages was found significant and the mean value of 0.59 for protein in case of freshly prepared beverages decreased to 0.49 and 0.51 stored up to 15 days on storage intervals (Figure 2). The significantly lowest protein was observed at 50% mango juice blended with paneer whey, followed by 30% and 40%. The total solid values

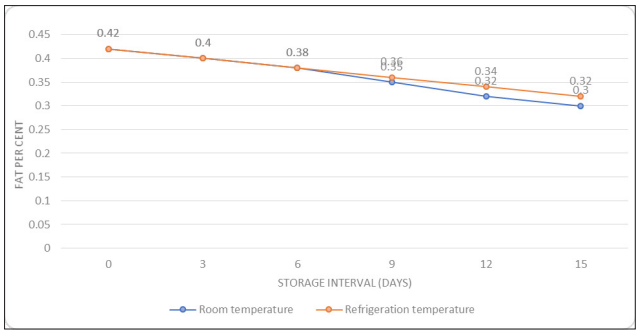


Figure 1: Effect of storage temperature and storage interval on fat percent of orange based paneer whey beverage.

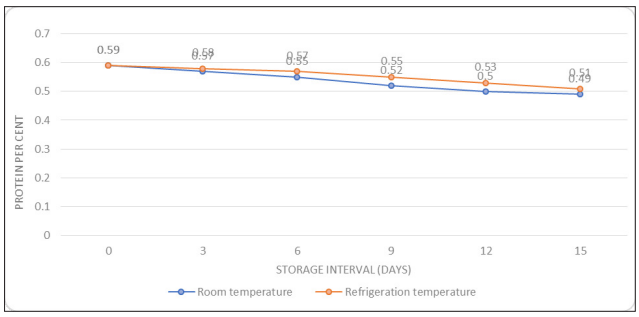


Figure 2: Effect of storage temperature and storage interval on protein percent of orange based paneer whey beverage.

decreased comparatively throughout the storage study period. The interaction between storage temperature and interval showed significantly lower protein levels. The differences between the periods of storage and conditions were compared by two-way ANOVA and the critical difference test as per Snedecor and Cochran.

Total sugar content

The data reveals that the storage period had a significant effect on the total sugar content of beverages at both room temperature (12.27%) and refrigeration temperature (12.44%) (Figure 3). The total sugar content decreased moderately faster in room temperature storage than in refrigeration storage. The interaction effect of storage interval and temperature combinations on total sugar percent of the product was statistically significant ($p < 0.05$). The differences between the periods of storage and conditions were compared by two-way ANOVA and the critical difference test as per Snedecor and Cochran. The mean total sugar content for treatment T_4 i.e. with 25% orange juice was decreasing in both storage conditions on the 3rd and last day.

Acidity content

The acidity was estimated to study the effect of different storage conditions and intervals on freshly prepared beverage treatment (T_4). The acidity percentage simultaneously increased with storage period, and the mean value of 0.32 in the freshly prepared beverage increased to 0.40 during 15 days of storage. The pooled data are presented in (Figure 4) showed that effect of different temperature and storage intervals on the mean score of acidity was significant and significantly changes were observed in acidity during the storage period.

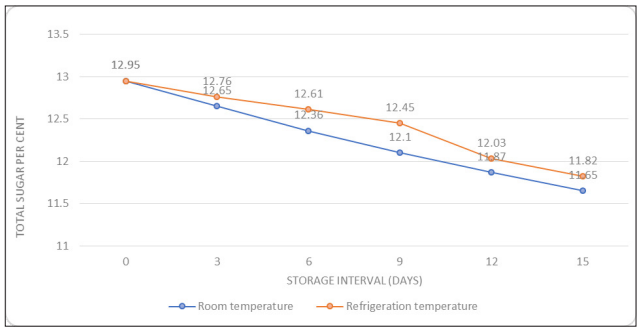


Figure 3: Effect of storage temperature and storage interval on total sugar percent of orange based paneer whey beverage.

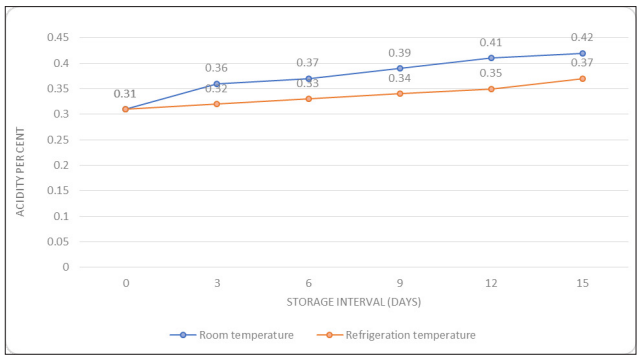


Figure 4: Effect of storage temperature and storage interval on acidity percent of orange based paneer whey beverage.

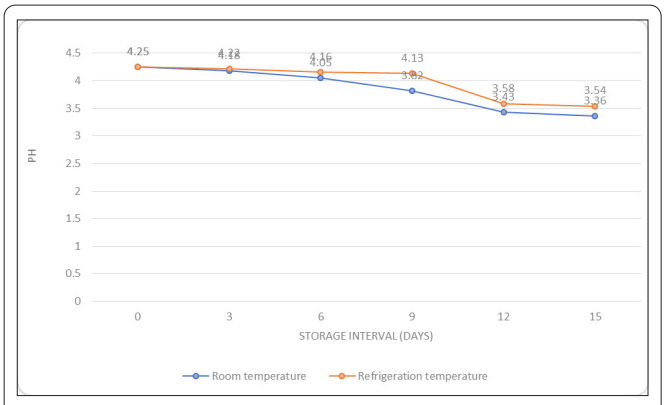


Figure 5: Effect of storage temperature and storage interval on pH of orange based paneer whey beverage.

pH

With regards to the pH of orange-based paneer whey beverage, it was noted that the individual means for the pH of paneer whey beverage prepared from blends of 75% paneer whey with 25% orange juice of treatment (T_4) under room temperature (28 ± 2 °C) and refrigeration temperature (7 ± 2 °C) were 3.85 and 3.97, respectively (Figure 5). The effect of different storage temperatures and storage intervals on the mean value of pH was significant ($p < 0.05$), and significant changes were observed in pH during the storage period. A decreasing trend was observed in pH with increasing acidity during 15 days of storage.

Microbiological characters

The results of the study indicated that the storage con-

ditions and intervals has statistically significant ($p < 0.05$) change in the colony of standard plate count and yeast and mold count when stored at room temperature condition. The standard plate count has increased significantly ($p < 0.05$) on successive room and refrigeration storage days from 0 to 15th days in whey-based orange beverages, including control (Table 3). The yeast and mould counts remained within the limit at refrigeration temperature (7 ± 2 °C). This finding agrees with the research work of Bhat and Singh [18] and Sakhale et al. [19]. However, the storage conditions x interval interaction for SPC and YMC were statistically significant ($p < 0.05$). The differences between the periods of storage and conditions were compared by two-way ANOVA and the critical difference test as per Snedecor and Cochran.

Discussion

Considering huge production and disposal problem of whey, the efforts were made through present investigation to assess the possibility of utilization of whey as by-product resulted from Indian dairy product i.e., paneer, for the manufacture of orange based paneer whey beverage. The present study is to utilize paneer whey for preparation of highly nutritious whey beverage by blending it with different levels of orange juice [19]. An attempt has been made in this investigation to evaluate for its physical and chemical analysis, sensory evaluation, storage life, microbial analysis. Scores of flavour, colour and appearance, consistency and overall acceptability of orange based paneer whey beverage (T_4) at refrigeration temperature (7 ± 2 °C) was appreciably slower rate of decrease than in the whey beverage stored at room temperature (28 ± 2 °C), respectively. Similar results have also been reported by Yadav et al. [20], who reported that the overall acceptability of the beverage was desirable up to 15 days of storage at refrigeration temperature. However, the sensory scores for flavour at refrigeration temperature were within acceptable range up to 9th days of prepared whey beverage.

The flavour of the beverage was mostly governed by highly concentrated, dark orange fruit juice. Considering the weighted score sensory analysis, the storage period significantly affected the sensory characteristics of the beverage, flavour, colour, consistency parameters ($p < 0.05$). Overall, the beverages were most acceptable on the 9th day of examination at refrigeration temperature and up to 3rd day at room temperature. According to the sensory evaluation, the acceptance slightly declined from day 3 and 9 onwards but significantly decreased on the last, 15th day of storage under room and refrigeration, respectively (Table 2). The most important factors for poor acceptability could be proliferation of micro-organisms and the decrease in pH. The colour as a sensory characteristics became less desirable after day 3 and 9. This can be linked to various leading to a shift in the particle size distribution, decreased sediment and enhanced brightness of the beverages, all of which lead to less appealing colour. Similar results were reported i.e., the decreased in flavour during storage could be possibly due to loss of volatile aromatic substances [21].

The changes in fat content during the 15 days of storage of orange-based paneer whey beverage decreased moderately faster in room temperature storage than in refrigeration storage temperature, irrespective of levels of incorporation of orange juice in paneer whey. A similar decline in fat was observed by Dubey et al. [22] in whey beverages. During the storage, the protein value of sample content of beverages was found significant ($p < 0.05$) and the mean value of 0.59 for protein in case of freshly prepared beverages decreased to 0.49 and 0.51 stored up to 15 days on storage intervals (Figure 2). It implies that, significant change in mean values of protein content was observed on storage of this product at room temperature as well as refrigeration temperature, respectively. The present result was in close agreement with the findings of Das et al. [23].

The storage period had a significant effect on the total sugar content of the beverage at both room temperature (12.27 %) and refrigeration temperature (12.44%) (Figure 3). These results are consistent with the findings reported by Yadav et al. [20], which showed a decreasing trend in total sugar content of whey-based mango RTS beverages (70:30) at refrigeration temperature over a 30-day period.

Table 3: Effect of room and refrigerated conditions on the microbiological characteristics of paneer whey-based orange juice beverage (T_4) (mean + SE).

Treatments	0-day	3-day	6-day	9-day	12-day	15-day
Standard plate count (CFU x 10^4)						
Room temperature	1.95	3.47	5.17	6.36	6.68	6.91
Refrigeration temperature	1.95	2.07	2.28	2.34	3.68	4.29
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.09		0.18	
Yeast and mould count (CFU x 10^2)						
Room temperature	0.14	0.83	1.23	1.33	2.44	3.32
Refrigeration temperature	0.14	0.33	0.44	0.6	0.65	0.74
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.15		0.31	

Note: SE ± (d)-Standard Error of Difference; CD-Critical Difference; A × B-Durations × Temperature condition; Sig.–Significant; Data are pooled mean of three replications. Significant ($p < 0.05$) variation between duration of the product and temperature conditions were assessed by F-Test.

During the storage period at various intervals, it was discovered that the acidity levels increased. This could be due to the conversion of lactose and protein into lactic acid and amino acids [21]. In another study by Dubey et al. [22], it is also observed that a similar relationship between acidity and pH, where acidity increased when acid whey produced from milk coagulation was used. In their study, acidity levels rose from 0.40 to 0.50 after 20 days of storage. Likewise, an increase in acidity was observed during the 21-day storage of orange juice whey drink.

The pH change during the 15-day storage of orange-based paneer whey beverage is documented in (Figure 5). Throughout the storage period, the pH value of the stored beverage remains relatively stable ($p < 0.05$), indicating no significant variation. However, there is a significant difference ($p < 0.05$) in the pH value for samples of the beverage stored under different conditions. During storage, the pH of the mixture significantly decreased ($p < 0.05$) due to the increase in titratable acidity values of the beverages. This decrease in pH aligns with the findings of a study Dubey et al. [22] on a whey-based watermelon drink, where the initial pH decreased from 5.1 to 5.05 after 30 days of storage. In another study conducted by Das et al. [23], it was observed that the pH values of a whey-based orange beverage decreased from 4.28 to 3.95. This decrease in pH during storage may be attributed to the citric acid and ascorbic acid present in the beverage, which interact with the sugar and protein components of the product. The production of organic acids and amino acids also contributes to increased acidity and a subsequent decrease in pH. However, the presence of protein and dietary fiber in the beverage may offer some protection for probiotics against the negative effects of pH, as previously reported for mango-based beverages [24]. The lower pH value of the beverage is beneficial for maintaining the stability of the final product during storage, as it inhibits the growth of harmful microorganisms, including coliforms [25].

Regardless of the type of beverage, the quantity of bacteria, yeast, and mold present increased progressively over time. The growth of these microorganisms followed a steady pattern during the storage period. Given the acidic nature of the beverages, it was unexpected to observe an increase in bacterial populations. Despite the beverages being manufactured in a sterile environment and stored at a temperature of 4 °C, the possibility of contamination cannot be completely ruled out. Statistically significant ($p < 0.05$) change in the colony of yeast and mould count was found when stored at room temperature condition. Bhat and Singh [18] and Sakhale et al. [19], found similar changes in yeast and mold counts in whey-based mango drink spreads stored at 4 + 1 °C for 30 days. The potential antimicrobial effects of stevia may have contributed to the highest bacterial count observed in the orange-based whey beverage [26-28].

The rise in yeast and mould counts in our research is consistent with the findings of the study Yadav et al. [20], although it contradicts the results reported by Ismail et al. [29], where yeast and mould counts decreased over time. The increase in microbial counts on day 1 and the subsequent storage period is not unexpected, since the beverages were made by mixing various ingredients (Table 3). However, the storage conditions x interval interaction for SPC and YMC were statistically significant ($p < 0.05$). The limited shelf life indicates that there is a chance for microbial contamination to occur again after the milk has been pasteurized. To enhance the preservation of the beverages, it is necessary to pasteurize the whey beverage using either traditional or innovative methods, considering the continuous growth of microorganisms.

Table 4: Effect of room and refrigerated conditions on sensory characteristics of paneer whey-based orange juice beverage (T_4) (mean + SE).

Treatments	0-day	3-day	6-day	9-day	12-day	15-day
Flavour						
Room temperature	8.23	6.84	5.64	5.33	5.02	4.38
Refrigeration temperature	8.23	7.6	6.4	6.01	5.36	5.05
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.1		0.3	
Colour and appearance						
Room temperature	8.27	6.88	5.87	5.4	4.95	4.41
Refrigeration temperature	8.27	7.67	7.22	6.93	5.62	5.1
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.15		0.46	
Consistency						
Room temperature	8.25	6.87	5.72	5.35	4.95	4.26
Refrigeration temperature	8.25	7.62	6.44	6	5.23	4.9
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.12		0.36	
Overall acceptability						
Room temperature	8.25	6.86	5.7	5.37	4.97	4.35
Refrigeration temperature	8.25	7.62	6.68	6.31	5.4	5.01
Interaction (A x B)	F-Test		SE ± (d)		CD at 5%	
	Sig.		0.1		0.3	

Note: SE ± (d)-Standard Error of Difference; CD-Critical Difference; A × B-Durations × Temperature condition; Sig.-Significant; Data are pooled mean of three replications. Significant ($p < 0.05$) variation between duration of the product and temperature conditions were assessed by F-Test.

In summary, whey is a good option to develop a functional orange-based whey drink. Therefore, the aim of the study was to formulate orange-based whey drinks using different mixtures of orange juice and paneer whey. In this study, a 25:75 blend of orange juice and paneer whey produced a high-quality beverage with a shelf life of 9 days under refrigerated conditions (7 + 2 °C) and 3 days at room temperature (28 + 2 °C) (Table 4).

Conclusion

The present study suggested that combined whey with fruits juices has the potential to create healthy beverages with desirable sensory characteristics. The study found that orange-based whey mixed beverages remained safe for consumption for up to 3 and 9 days when stored under room and refrigerated conditions. Considering the functional properties of the bioactive constituents found in fruit and whey, it is believed that orange whey based mixed beverages could be a valuable product in the growing beverage industry. However, further research is needed to determine shelf life, improve quality using stabilizers and opaque packaging, optimize process conditions, and conduct clinical trials to ensure safety and confirm the beverage's health benefits.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

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