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Development of probiotic rich whey beverage infused with lactic acid bacteria and fresh orange juice

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ABSTRACT

This study was conducted to develop an orange flavored whey based probiotic beverage using Lactobacillus acidophilus and Lactobacillus plantarum with evaluation of its physico -chemical properties and analysis of product stability during storage at room storage. Seven blends of drinks were prepared using varying concentration of whey and orange juice which were coded as T1 (100% whey & 0% orange juice, considered as the control sample), T2 (75% whey & 25 % orange juice), T3 (50% whey & 50 % orange juice) and T4 (25% whey & 75% orange juice), using Lactobacillus acidophilus as probiotic; while list T5(75%& 25 % orange juice), T6(50% whey & 50% orange juice), T7 (25% whey & 75 % orange juice) using Lactobacillus plantarum as probiotic starter culture with all blends containing 8% sugar. Our results showed that all variants were pleasant in overall acceptability while 25:75 blends of whey and orange juice (T4 & T7) showed highest sensory score however, the T7 blend containing Lactobacillus plantarum showed a little higher acceptance than the T4 blend containing Lactobacillus acidophilus. The physio-chemical analysis revealed that pH value, TSS, lactic acid(%), reducing sugar and total sugar decreased with storage, while titratable acidity and antioxidant properties decreased with storage. Viability of Lactobacilli remained high till 45 days and thereafter began to decline. Conclusively, whey and orange based probiotic beverage can serve as a desirable functional beverage.

Keywords: *Lactobacillus acidophilus, Lactobacillus plantarum, Sensory quality, Storage stability, Probiotic beverage, Antimicrobial activity, Antioxidant property.*

Introduction

Gut health is essential in a day sedentary lifestyle. As the fast moving society rhythm of modern life, shifts in dietary habits have become a common feature of urban living. More people are increasingly seeking healthier nutrition, making it priority in this era of globalization. Among the many options, probiotic-flavoured beverages are becoming increasingly popular, valued for both their pleasant taste and health benefits, as well as the ease with which they can be stored and consumed. Although probiotics have long been used in a variety of dairy-based foods, issues such as lactose intolerance and cholesterol concerns have encouraged the development of non-dairy probiotic products. With the right formulation, probiotic strains can be successfully added to appealing food items while still retaining their beneficial activity. In addition, fermentation serves as an effective method to improve the beverage's palatability while simultaneously extending its shelf life. Increasing awareness amongst people regarding the relationship between fermented food and health have thus opened a new dimension for so-called “functional foods” in recent years. Lactic acid bacteria (LAB) play a vital role in the fermentation process due to their specialized enzymatic systems. These systems release extracellular proteases and lipase, which break down complex proteins, lipids, and carbohydrates into simpler compounds, thereby accelerating and enhancing fermentation Zhong *et al.,*[1]. Among these, *Lactobacillus acidophilus* and *Lactobacillus plantarum* are widely recognized as novel probiotic strains with significant functional benefits. Whey, a nutrient-rich by-product obtained during the manufacture of dairy products such as cheese, chhana, sweets, and paneer, is another valuable component in this context. Despite its nutritional value, whey disposal remains a major concern for the dairy industry because of its high biological oxygen demand (BOD), which typically ranges from 39,000 to 48,000 ppm Ryan *et al.,* [2]. Whey-based lactic beverages are an effective way to make use of liquid whey, as they combine high nutritional value with low production cost and good sensory appeal. In preparing such beverages, different lactic acid bacteria (LAB) and yeasts can be employed. Citrus fruits, with their fresh and tangy flavor, add to the taste and consumer acceptability of these products. Orange juice, in particular, provides a rich source of vitamin C and helps to mask the slight bitterness often associated with whey. Despite these advantages, there are relatively few studies on the use of citrus fruit by-products, especially orange juice as a medium for isolating probiotic LAB strains and supporting their fermentation in natural juice Pérez *et al.,* [3]. This research aimed to develop a probiotic whey–orange juice beverage containing viable cells of *Lactobacillus acidophilus* and *Lactobacillus plantarum* under ambient storage conditions. The survival of both probiotic cultures, along with physicochemical attributes such as pH, total soluble solids (TSS), titratable acidity, lactic acid, reducing sugars, total sugars, ascorbic acid content, antioxidant activity, and sensory properties, were evaluated at regular intervals over a 45-day storage period.

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Materials and Methods

Probiotic bacterial strains:

The probiotic culture *Lactobacillus acidophilus* (MTCC 10307) and *Lactobacillus plantarum* (MTCC 12062) were obtained from IMTECH Chandigarh in lyophilized form.

For activation, both cultures were inoculated into sterilized skim milk medium and incubated at 37 °C for 24 hours Gomes *et al.,*[4].

Preparation of probiotic beverage:

Seven different beverages were prepared by different blends of whey and orange juice coded as T1 (100% whey & 0% orange juice, considered as the control sample), T2 (75% whey & 25 % orange juice + *Lactobacillus acidophilus*), T3 (50% whey & 50 % orange juice + *Lactobacillus acidophilus*) and T4 (25% whey & 75% orange juice + *Lactobacillus acidophilus*), T5(75% & 25 % orange juice + *Lactobacillus plantarum*), T6 (50% & 50% orange juice + *Lactobacillus plantarum*), T7 (25% & 75 % orange juice + *Lactobacillus plantarum*) and all formulations contained 8% sugar. Five ml of activated *Lactobacillus acidophilus* (1.5 x 10^8 cfu/ml) and *Lactobacillus plantarum* (1.5 x 10^8 cfu/ml) was inoculated into 500 ml of pasteurized fruit juice and whey blends in sterilized glass bottles. Finally, the inoculated blends made were incubated at 37°C for 5 h Shukla *et al.,*[5].

Physico-chemical analysis:

Various parameters including pH (measured using a pH meter), total soluble solids (TSS; measured by refractometer), titratable acidity (determined by titration), lactic acid (determined by titration method), reducing sugars (analyzed using the Lane and Eynon volumetric method), total sugars (determined through acid hydrolysis followed by titration with Fehling's solution using phenolphthalein as an indicator), and ascorbic acid content (estimated by titration with 2,6-dichlorophenolindophenol dye) were evaluated to assess the effect of fermentation duration and storage time on the formulated probiotic beverage.

Total antioxidant activity of the developed probiotic beverages

Antioxidant activity DPPH (%):

The radical scavenging activity of the extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was assessed using a spectrophotometric method as described by a previous study. In this assay, 2 ml of a freshly prepared ethanolic DPPH solution was mixed with varying concentrations (40, 60, 80, and 100 µg/ml) of beverage extracts in separate test tubes. The mixtures were vigorously shaken and was incubated at 37°C for 30 minutes in the dark to allow the reaction to occur. After incubation, the absorbance of each solution was measured at 517 nm using a UV-Vis spectrophotometer.

The percentage of radical scavenging activity (RSA%) was calculated using the following formula:

$RSA (\%) = (A_0 - A_1 / A_0) \times 100$

where:

- A_0 is the absorbance of the control (without extract)
- A_1 is the absorbance of the sample (with extract)

The concentration of extract required to inhibit 50% of the DPPH radicals (IC_{50} value) were determined by plotting the percentage inhibition against the extract concentrations and calculating the corresponding value from the graph Kosanic *et al.,*[6].

Total phenols (mg GAE/100g):

The total phenolic content of the extract was determined using the Folin–Ciocalteu method as outlined by Singleton and Rossi (1965). Different concentrations of the sample were prepared by dissolving the extract in distilled water. Two millilitres of Folin–Ciocalteu reagent (previously diluted with distilled water) were added, and the mixture was incubated at 37 °C for 10 minutes. Subsequently, 3 ml of sodium carbonate solution was added, the mixture was gently vortexed for 2 minutes, and then kept in a dark chamber for 30 minutes to allow the reaction to proceed. After incubation, absorbance was measured at 760 nm using a UV–Visible spectrophotometer. The total phenolic content was calculated from a gallic acid calibration curve and expressed as milligrams of gallic acid equivalents (mg GAE/100 g sample) Alispahić *et al.,* [7].

Microbial analysis: Total plate count (CFU/g):

The total viable count (TVC) (Log_{10} cfu/ml) was determined using the standard plate count method as described by Vanderzant *et al.,* [8]. Plate count agar and a sterile saline solution (0.9% NaCl w/v) were first prepared. Then, 1 ml of each sample was transferred into the saline solution, and serial dilutions were performed up to 10^{-5}. From each dilution, an aliquot was transferred to sterile Petri dishes, followed by the addition of 10–15 ml of molten agar. The plates were gently revolved to ensure even distribution and left to solidify. Once set, they were incubated at 32 °C for 48 hours. After incubation, colonies were counted on plates containing 30–300 colonies, and each dilution was plated in duplicate to improve accuracy.

Sensory evaluation

Sensory evaluation was performed using a 9-point hedonic scale. Evaluation were presented. The mean scores for the whey-orange beverages showed significant differences ($p < 0.05$) in terms of color, appearance, aroma, and overall appearance. All the beverage types received similar scores for color, appearance, and flavor, except for type D (35% whey and 65% orange juice), which was rated lower by Shukla *et al.,* [5]. reported that the highest flavor score was observed in the beverage containing 65% whey and 35% orange juice, compared to other blend ratios.

Statistical analysis

For statistical analysis, the mean method was applied. The arithmetic mean of the replication values was calculated, after which the maximum and minimum values for all microbial counts, physico-chemical parameters, and nutritional values during storage were determined.

Results and Discussion

The results are given and discussed in the next section

Development of probiotic whey-orange beverage

The probiotic beverages was prepared by blending whey and orange juice in different proportions i.e. T1 (100% whey & 0% orange juice, considered as the control sample), T2 (75% whey & 25 % orange juice + *Lactobacillus acidophilus*), T3 (50% whey & 50 % orange juice + *Lactobacillus acidophilus*) and T4 (25% whey & 75% orange juice + *Lactobacillus acidophilus*), T5(75% & 25 % orange juice + *Lactobacillus plantarum*), T6 (50% & 50% orange juice + *Lactobacillus plantarum*), T7 (25% & 75% orange juice + *Lactobacillus plantarum*). The prepared samples were packed into glass bottles and kept at room temperature for storage.

These findings are consistent with the observations in agreement with Idan *et al.,*[9] and AbdulAlim *et al.,* [10] who confirmed that whey-fruit blends improve probiotic viability, antioxidant activity, and sensory acceptance. Thus, the beverages developed in this study demonstrate potential as functional health drinks with probiotic benefits.

Physio- chemical changes during storage of developed probiotic beverages

The variations in pH, total soluble solids (TSS), titratable acidity (TA), and lactic acid content of probiotic whey–orange juice beverages during storage are presented in Table 1. A steady decline in pH was observed in all treatments as storage progressed. The lowest mean pH was found in T7 (3.42), closely followed by T4 (3.47), whereas T1 recorded the highest value (4.07). This drop in pH can be explained by the activity of probiotic cultures (*Lactobacillus acidophilus* and *Lactobacillus plantarum)*, which convert sugars into organic acids during fermentation. Similar trends have been reported by Shukla *et al.,*[5] who noted a gradual fall in pH in fermented whey–fruit beverages over time.

A gradual decrease in TSS was observed during storage, most likely due to the consumption of sugars by lactic acid bacteria for acid production. Among the treatments, the highest mean TSS was recorded in T7 (12.11 °Brix), followed by T4 (11.82 °Brix), whereas the lowest was noted in T1 (9.54 °Brix).The TSS of the probiotic beverage decreased with an increase in the proportion of orange juice. In according to findings of this study Bakuradze *et al.,*[11] and Deshpande *et al.,* [12] also reported similar drops in TSS during storage of probiotic orange drinks.

Titratable acidity showed a significant increase over the storage period, indicating greater production of organic acids. The highest mean value was recorded in T7 (0.95%), closely followed by T4 (0.93%), while T1 had the lowest (0.56%). This pattern was in line with the pH results, where higher acid production was associated with a lower pH value.These results are supported by earlier findings of Shukla and Kushwaha [13] found acidity increase with more orange juice in whey drinks.

Lactic acid content also increased steadily during storage, with T7 recording the highest mean value (0.76%), followed by T4 (0.74%), while T1 remained the lowest at 0.44%. The higher lactic acid levels in the orange juice–rich treatments suggest enhanced growth and metabolic activity of *Lactobacillus plantarum* and *Lactobacillus acidophilus*, likely supported by the greater availability of fermentable sugars and the favourable pH buffering capacity provided by the orange juice. Pérez *et al.,* [3] reported that mixing *L. brevis* and *L. plantarum* with orange juice and milk produced a lot of acid, which shows that these strains are very effective at fermenting fruit juice

The changes in reducing sugar, total sugar, and ascorbic acid content during storage of probiotic whey–orange juice beverages are shown in Table 2. Reducing sugar content declined gradually across all treatments during storage. The highest mean value was recorded in T7 (5.43%), followed by T4 (5.23%), while T1 had the lowest (3.92%). This reduction can be explained by the utilisation of reducing sugars by probiotic bacteria for organic acid synthesis. Formulations with more orange juice (T4, T7) retained higher levels due to their greater initial sugar content and Total sugar levels decreased significantly ($P<0.01$) over time. T7 recorded the highest mean (7.75%), followed by T4 (7.57%), while T1 had the lowest (5.14%). The decline reflects microbial fermentation, where sugars are metabolised for energy and acid production.

Orange juice–rich treatments maintained higher levels due to their higher starting carbohydrate content. These findings are in line with Deshpande *et al.,* [12].

Ascorbic acid content decreased progressively during storage, consistent with its susceptibility to oxidation. The highest mean value was observed in T7 (48.65 mg/100 ml), followed by T4 (47.38 mg/100 ml), whereas T1, containing only whey, had the lowest (0.93 mg/100 ml). Higher retention in orange juice–based treatments is due to their greater initial vitamin C content and the possible protective effects of juice components. The results are similar to those of Shukla and Kushwaha [13], who found that as the probiotic beverage developed from orange juice and whey was stored in the refrigerator, the amount of ascorbic acid gradually decreased.

The antioxidant potential of the probiotic beverages, determined through DPPH radical scavenging activity, revealed that % inhibition increased with orange juice content. Beverages fermented with *Lactobacillus acidophilus* showed DPPH inhibition ranging from 21.80% to 27.40% across concentrations, while those with *Lactobacillus plantarum* ranged from 29.10% to 33.60%. The highest antioxidant activity was observed in T7 (25:75 W: OJ: LP) with the lowest IC_{50} value of 43.7 μg, indicating strong radical scavenging potential (Fig. 2). These results align with Zeng *et al.,*[14], who found that after 48-h fermentation, orange juice exhibited the best DPPH and ABTS scavenging capacities among five juices tested. Sharoba *et al.,* [15] reported enhanced antioxidant activity in orange juice inoculated with *Lactobacillus acidophilus* and *Lactobacillus plantarum* compared to non-inoculated control (Fig. 2).

Similarly, the Total Phenolic Content (TPC) increased with orange juice proportion and probiotic activity. The TPC of beverages fermented with *Lactobacillus acidophilus* ranged from 27.20 to 42.30 μg, while those fermented with *Lactobacillus plantarum* ranged from 30.60 to 45.08 μg across concentrations (Fig.3) . The highest TPC was recorded in T7 (25:75 W: OJ: LP), reflecting the synergistic effect of orange juice phenolics and probiotic fermentation. These findings, Bakuradze *et al.,,* [11] suggest that both the orange juice content and the probiotic strain used significantly influenced the antioxidant capacity and phenolic composition of the probiotic beverages.

Effect of fermentation on sensory evaluation of developed probiotic beverage

In all the probiotic beverages fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarum,* the hedonic scale points representing the average score of its different parameters like color, appearance, aroma and overall acceptability have been presented in Tables 3. The results revealed that color, appearance, aroma and overall acceptability of the probiotic beverage (25:75) gets the best result in both fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarum.* The results above declare from physico-chemical and sensory evaluation, that only probiotic beverage T4 and T7 (25:75) has shown thebest results from both the bacterial species fermented samples. Therefore, for shef-life study only the probiotic beverage (25:75) has been taken. These findings are in agreement with Deshpande et al., [12] and Shukla and Kushwaha [13] who found that increasing the proportion of fruit juice in probiotic beverages enhanced their visual appeal.

Microbial analysis total plate count (cfu/ml)

The storage study showed that the developed whey–orange juice probiotic beverages maintained a healthy level of probiotic bacteria throughout 45 days of storage, with gradual but expected changes over time (Table 4). In the 25:75 whey–orange juice formulations, T4 containing *Lactobacillus acidophilus* started with 8.60 ± 0.02 log cfu/ml on day 5, while T7 with *Lactobacillus plantarum* began at 8.70 ± 0.01 log cfu/ml. Over the storage period, both treatments showed a slow decline in counts, reaching 7.20 ± 0.02 and 7.40 ± 0.02 log cfu/ml respectively by day 45. This decline is a natural result of acid stress and nutrient depletion during storage, a trend also seen in earlier studies on probiotic fruit-based beverages kumar *et al.,* [16]. Importantly, even after 45 days, both remained well above the recommended minimum of 6 log cfu/ml, ensuring the beverages retained their probiotic benefits. No unwanted bacterial growth was detected in either T4 or T7 during the first 15 days. From day 30 onwards, only low bacterial counts appeared—1.05×10^8 and 1.00×10^8 cfu/ml for T4 at days 30 and 45, and 0.76×10^8 and 0.70×10^8 cfu/ml for T7 in the same period. These low values suggest that the beverage environment was supportive of the probiotic cultures but not favorable for spoilage or contaminating bacteria. Fungal growth (yeasts and molds) was absent in both treatments for the entire storage period. This stability is likely due to the combined effects of low pH, organic acid production, and antimicrobial compounds produced by the probiotic strains, such as hydrogen peroxide and bacteriocins.

Fig. 1: Probiotic Beverages

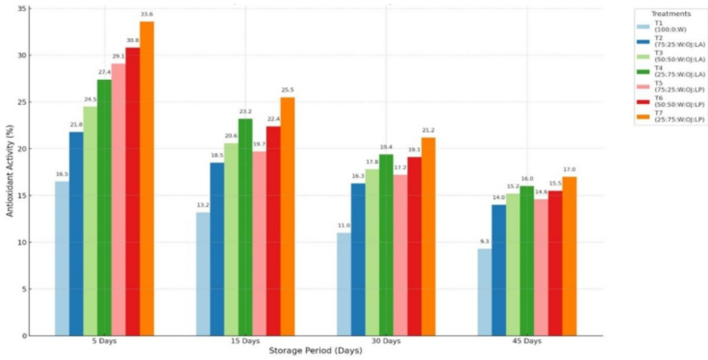

Fig. 2: Graphical representation of DPPH of developed probiotic beverage

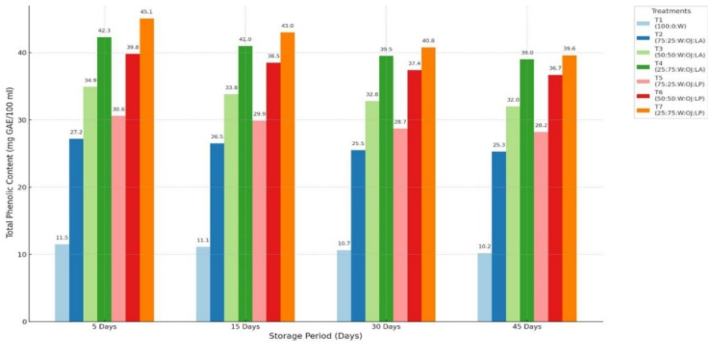

Fig. 3: Graphical representation of total phenolic content of developed probiotic beverages

Table 1: Formulation of probiotic whey-orange juice beverages with different levels of whey, orange juice, and probiotic culture

Treatment	Whey (%)	*Lactobacillus acidophilus* (1.5 x 10^8 CFU/ml)	*Lactobacillus plantrum* (1.5x10^8CFU/ml)	Orange juice (%)
T1	100	-	-	-
T2	75	5 ml	-	25
T3	50	5 ml	-	50
T4	25	5 ml	-	75
T5	75	-	5 ml	25
T6	50	-	5 ml	50
T7	25	-	5 ml	75

Table2: Effect of treatments and storage period on pH and TSS of developed probiotic beverages

TREATMENTS	pH					TSS				
	STORAGE PERIOD (DAYS)					STORAGE PERIOD (DAYS)				
	Day 5	Day 15	Day 30	Day 45	Mean	Day 5	Day 15	Day 30	Day 45	Mean
T1 (100:0 Whey)	4.20	4.11	4.02	3.93	**4.07**	10.00	9.66	9.52	8.98	**9.54**
T2 (75:25 W:OJ:LA)	3.90	3.86	3.77	3.68	**3.80**	11.42	11.08	10.74	10.40	**10.91**
T3 (50:50 W:OJ:LA)	3.75	3.66	3.57	3.48	**3.62**	11.90	11.56	11.45	10.88	**11.45**
T4 (25:75 W:OJ:LA)	3.60	3.51	3.42	3.33	**3.47**	12.72	11.98	11.60	10.96	**11.82**
T5 (75:25 W:OJ:LP)	3.85	3.76	3.74	3.58	**3.73**	11.62	11.28	10.94	10.70	**11.14**
T6 (50:50 W:OJ:LP)	3.70	3.61	3.52	3.43	**3.57**	12.00	11.66	11.52	10.98	**11.54**
T7 (25:75 W:OJ:LP)	3.55	3.46	3.37	3.28	**3.42**	13.08	12.34	11.98	11.05	**12.11**
MEAN	**3.79**	**3.71**	**3.63**	**3.53**		**11.82**	**11.51**	**11.39**	**10.71**	
CD (P<0.01)	Treatment(T): 0.06; Storage(S): 0.05; (T)X(S): 0.07					Treatment(T): 0.06; Storage(S): 0.05; (T)X(S): 0.07				

Table 3: Effect of treatments and storage period on Titratable acidity and Lactic acid of developed probiotic beverages

TREATMENTS	Titratable Acidity					Lactic Acid				
	STORAGE PERIOD (DAYS)					STORAGE PERIOD (DAYS)				
	Day 5	Day 15	Day 30	Day 45	Mean	Day 5	Day 15	Day 30	Day 45	Mean
T1 (100:0 Whey)	0.50	0.55	0.58	0.60	**0.56**	0.40	0.44	0.46	0.48	**0.44**
T2 (75:25 W:OJ:LA)	0.70	0.78	0.86	0.92	**0.82**	0.56	0.62	0.69	0.74	**0.65**
T3 (50:50 W:OJ:LA)	0.75	0.82	0.93	0.98	**0.87**	0.60	0.66	0.74	0.78	**0.70**
T4 (25:75 W:OJ:LA)	0.78	0.90	0.97	1.05	**0.93**	0.62	0.72	0.78	0.84	**0.74**
T5 (75:25 W:OJ:LP)	0.74	0.82	0.90	0.95	**0.85**	0.59	0.66	0.72	0.76	**0.68**
T6 (50:50 W:OJ:LP)	0.77	0.88	0.98	1.00	**0.91**	0.62	0.70	0.78	0.80	**0.72**
T7 (25:75 W:OJ:LP)	0.82	0.92	1.00	1.06	**0.95**	0.66	0.74	0.80	0.85	**0.76**
MEAN	**0.72**	**0.81**	**0.89**	**0.94**	–	**0.58**	**0.65**	**0.71**	**0.75**	
CD (P<0.01)	Treatment(T): 0.02; Storage(S): 0.02; (T)X(S): 0.02					Treatment(T): 0.04; Storage(S): 0.03; (T)X(S): 0.06				

Table 4: Effect of treatments and storage period on Reducing sugar and Total sugar of developed probiotic beverages

TREATMENTS	Reducing sugar					Total sugar				
	STORAGE PERIOD (DAYS)					STORAGE PERIOD (DAYS)				
	Day 5	Day 15	Day 30	Day 45	Mean	Day 5	Day 15	Day 30	Day 45	Mean
T1 (100:0 Whey)	4.29	4.00	3.79	3.60	**3.92**	6.09	5.20	4.81	4.46	**5.14**
T2 (75:25 W:OJ:LA)	5.1	4.55	3.88	3.25	**4.20**	6.94	5.95	5.56	5.0	**5.86**
T3 (50:50 W:OJ:LA)	5.75	5.09	4.76	3.56	**4.79**	8.20	6.94	5.81	5.50	**6.61**
T4 (25:75 W:OJ:LA)	6.00	5.6	4.99	4.36	**5.23**	8.93	7.81	6.94	6.58	**7.57**
T5 (75:25 W:OJ:LP)	5.28	4.65	3.90	3.30	**4.28**	6.97	5.96	5.62	5.10	**5.91**
T6 (50:50 W:OJ:LP)	5.95	5.28	4.86	3.69	**4.95**	8.62	7.10	6.58	5.95	**7.06**
T7 (25:75 W:OJ:LP)	6.11	5.78	5.05	4.76	**5.43**	9.26	8.06	6.98	6.7	**7.75**
MEAN	**5.29**	**4.96**	**4.32**	**3.71**		**7.57**	**6.43**	**5.90**	**5.47**	
CD (P<0.01)	Treatment(T): 0.06; Storage(S): 0.04; (T)X(S): 0.09					Treatment(T): 0.08; Storage(S): 0.05; (T)X(S): 0.16				

Table 5: Effect of treatments and storage period on ascorbic acid of developed probiotic beverages

TREATMENTS	STORAGE PERIOD (DAYS)				
	Day 5	15 Days	30 Days	45 Days	Mean
T1 (100:0 WHEY)	1.00	0.95	0.9	0.85	**0.93**
T2 (75:25 W:OJ:LA)	37.5	35.9	34.3	32.8	**35.13**
T3 (50:50 W:OJ:LA)	44.1	42.6	41.1	39.7	**41.88**
T4 (25:75 W:OJ:LA)	50.8	48.5	46.2	44.0	**47.38**
T5 (75:25 W:OJ:LP)	38.9	37.4	35.9	34.4	**36.65**
T6 (50:50 W:OJ:LP)	45.5	43.9	42.4	40.9	**43.18**
T7 (25:75 W:OJ:LP)	52.1	49.8	47.5	45.2	**48.65**
Mean	**38.56**	**37.29**	**35.2**	**34.84**	
CD (P<0.01)	Treatment(T): 0.42; Storage(S): 0.28; (T)X(S): 0.78				

Table 6: Effect of treatments and storage period on Colour and Appearance of developed probiotic beverages

TREATMENTS	Colour					Appearance				
	STORAGE PERIOD (DAYS)					STORAGE PERIOD (DAYS)				
	Day 5	Day 15	Day 30	Day 45	Mean	Day 5	Day 15	Day 30	Day 45	Mean
T1 (100:0 Whey)	7.38	7.12	7.01	6.98	**7.12**	7.21	7.07	6.93	6.82	**7.00**
T2 (75:25 W:OJ:LA)	8.10	7.88	7.67	7.58	**7.81**	7.83	7.70	7.62	7.48	**7.65**
T3 (50:50 W:OJ:LA)	8.50	8.38	8.25	8.00	**8.28**	8.35	8.23	8.11	8.05	**8.18**
T4 (25:75 W:OJ:LA)	8.70	8.53	8.45	8.09	**8.44**	8.49	8.37	8.23	8.10	**8.44**
T5 (75:25 W:OJ:LP)	8.20	7.98	7.80	7.7	**7.92**	8.00	7.86	7.67	7.58	**7.77**
T6 (50:50 W:OJ:LP)	8.65	8.43	8.30	8.05	**8.36**	8.57	8.45	8.30	8.07	**8.35**
T7 (25:75 W:OJ:LP)	8.73	8.63	8.58	8.15	**8.52**	8.65	8.5	8.37	8.20	**8.43**
MEAN	**8.32**	**8.14**	**8.01**	**7.79**		**8.15**	**8.02**	**7.89**	**7.75**	
CD (P<0.01)	Treatment(T): 0.06; Storage(S): 0.05; (T)X(S): 0.07					Treatment(T): 0.04; Storage(S): 0.03; (T)X(S): 0.07				

Table 7: Effect of treatments and storage period on Aroma and Overall acceptability of developed probiotic beverages

TREATMENTS	Aroma					Overall acceptability				
	STORAGE PERIOD (DAYS)					STORAGE PERIOD (DAYS)				
	Day 5	Day 15	Day 30	Day 45	Mean	Day 5	Day 15	Day 30	Day 45	Mean
T1 (100:0 Whey)	7.36	7.20	7.08	6.98	**7.16**	7.13	6.98	6.85	6.70	**6.91**
T2 (75:25 W:OJ:LA)	8.15	7.91	7.80	7.63	**7.87**	7.98	7.77	7.60	7.50	**7.69**
T3 (50:50 W:OJ:LA)	8.38	8.25	8.19	7.98	**8.20**	7.67	7.54	7.40	7.15	**7.44**
T4 (25:75 W:OJ:LA)	8.50	8.43	8.23	8.07	**8.31**	7.98	7.68	7.26	7.81	**7.68**
T5 (75:25 W:OJ:LP)	8.20	7.93	7.83	7.70	**7.92**	8.09	7.90	7.76	7.63	**7.84**
T6 (50:50 W:OJ:LP)	8.70	8.53	8.37	8.10	**8.43**	8.31	8.16	8.01	7.98	**8.08**
T7 (25:75 W:OJ:LP)	8.85	8.78	8.56	8.25	**8.61**	8.50	8.36	8.28	8.01	**8.28**
MEAN	8.30	8.14	8.00	7.81		7.95	7.77	7.58	7.52	
CD (P<0.01)	Treatment(T): 0.02; Storage(S): 0.01; (T)X(S): 0.04					Treatment(T): 0.03; Storage(S): 0.02; (T)X(S): 0.05				

Table 8: Probiotic cell viability (cfu /ml) and other microbes in developed probiotic beverages

Treatment	Storage	Probiotic LAB culture	Total plate count (=10^8 x cfu/ml)	Fungus 10(=10^6 x cfu/ml)	
T1 (100:0::whey)	5 DAY	8.42 ± 0.02	NIL	NIL	
	15 DAY	8.10 ± 0.03	NIL	NIL	
	30 DAY	7.65 ± 0.03	1.30 × 10^8	0.55 × 10^3	
	45 DAY	6.80 ± 0.02	1.28 × 10^8	0.53 × 10^3	
T2(75: 25::W: OJ:LA)	5 DAY	8.50 ± 0.01	NIL	NIL	
	15 DAY	8.20 ± 0.02	NIL	NIL	
	30 DAY	8.20 ± 0.02	1.20 × 10^8	0.50 × 10^3	
	45 DAY	8.20 ± 0.02	1.18 × 10^8	0.48 × 10^3	
T3(50: 50:W: OJ:LA)	5 DAY	8.60 ± 0.01	NIL	NIL	
	15 DAY	8.30 ± 0.01	NIL	NIL	
	30 DAY	7.85 ± 0.02	1.12 × 10^8	0.46 × 10^3	
	45 DAY	7.85 ± 0.02	1.10 × 10^8	0.44 × 10^3	
T4(25: 75:W: OJ:LA)	5 DAY	8.48 ± 0.02	NIL	NIL	
	15 DAY	8.15 ± 0.02	NIL	NIL	
	30 DAY	8.15 ± 0.02	1.05 × 10^8	NIL	
	45 DAY	8.15 ± 0.02	1.00 × 10^8	NIL	
T5(75: 25::W: OJ:LP)	5 DAY	8.60 ± 0.01	NIL	NIL	
	15 DAY	8.30 ± 0.02	NIL	NIL	
	30 DAY	7.90 ± 0.02	0.95 × 10^8	0.38 × 10^3	

	45 DAY	7.25 ± 0.02	0.90×10^8	0.36×10^3	
T6(50: 50:W: OJ:LP)	5 DAY	8.70 ± 0.02	NIL	NIL	
	15 DAY	8.40 ± 0.01	NIL	NIL	
	30 DAY	8.00 ± 0.03	0.85×10^8	0.35×10^3	
	45 DAY	8.00 ± 0.03	0.80×10^8	0.34×10^3	
T7(25: 75:W: OJ:LP)	5 DAY	8.55 ± 0.01	NIL	NIL	
	15 DAY	8.25 ± 0.02	NIL	NIL	
	30 DAY	7.80 ± 0.02	0.75×10^8	NIL	
	45 DAY	7.10 ± 0.02	0.70×10^8	NIL	

Conclusion

This study showed that a 25:75 blend of whey and orange juice can be turned into a tasty, healthy probiotic drink. Both *Lactobacillus acidophilus* and *Lactobacillus plantarum* stayed active and beneficial for 45 days at room temperature, but the version made with *Lactobacillus plantarum* (T7) held its quality a bit better—keeping more nutrients, showing stronger antioxidant activity, and scoring higher in taste tests. Overall, the *Lactobacillus plantarum* beverage proved to be a nutritious, shelf-stable, and enjoyable probiotic option.

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Conflict of Interest

Authors declare no competing interest.

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