PROBIOTIC BLENDED KINNOW JUICE

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ABSTRACT-Fruit juices are considered a good medium for carrying probiotic cells, as they inherently contain beneficial nutrients and have a pleasant taste. Kinnow, Pomegranate and Ginger juice was blended in the ratio of 89:10:1, respectively. Two probiotic cultures *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *lactis*, were added to the blended Kinnow juice, individually as well as in combination, with an initial concentration of 10^{12}-10^{13} CFU/ml. The probiotic viability, pH, TSS, titrable acidity, total sugars, vitamin C and microbial contaminants were determined by analyzing the developed probiotic juices at weekly intervals. The probiotic count was found higher in case of juice, containing *L. acidophilus* individually. Sensory evaluation revealed a consumer preference for blended Kinnow juice containing probiotic cells of *L. acidophilus* with a mean score for overall acceptability of 8.3. Determination of microbial contaminants (Total Plate Count, Yeast and Mould count, *E. coli* count and Coliforms) was done and these were found to be absent till five weeks of refrigerated storage. Coliforms and *E. coli* were completely absent throughout the storage period of seven weeks.

Keywords: Probiotic, blended Kinnow juice, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *lactis*, viability

Abbreviations
LA – *Lactobacillus acidophilus*
LD – *Lactobacillus delbrueckii* subsp. *lactis*
LA+LD - *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis*

INTRODUCTION

Consumers prefer foods that promote overall well being of an individual. The food with functional properties, have fulfilled this demand of consumers and the food industry. In mid-1980s, Japanese government had introduced the term "functional foods" [1]. Probiotics are defined as "Live microorganisms which have useful effects on host by improving the intestinal microbial balance" [2]. According to FAO/WHO [3], probiotics are defined as live microorganisms, which when ingested in definite numbers exert benefits to the health beyond inherent general nutrition. Many effects of probiotics have been reported on the health of an individual such as cholesterol reduction, reduction of blood ammonia levels, stimulation of the immune system, diabetes prevention, treatment and prevention of rotavirus diarrhea, for restoration of normal intestinal microflora after antibiotic therapy, anti-carcinogenic and anti-mutagenic effects and increasing lactose tolerance [4].

An ideal probiotic strain should not lose its viability or have any negative effect on the sensory properties of the food product during the process of manufacturing. Fermentative dairy products are considered as good carrier substrates for probiotic microorganisms into the human digestive tract, but lactose intolerance and cholesterol content are the two major drawbacks related to consumption of these products [5, 6]. Therefore, non-dairy probiotic products are desirable like fruit and vegetable juices.

Kinnow is a variety of citrus fruit cultivated broadly in the Punjab Province of both India and Pakistan. It is a hybrid of two citrus cultivators – "King" (*Citrus nobilis*) × "Willow Leaf" (*Citrus deliciosa*), first developed at the Citrus Research Centre of the University of California, Riverside, USA [7]. It contains citric acid, vitamins mostly vitamin C, vitamin A, minerals such as iron, phosphorus and has calcium also. Kinnow juice can be blended with Pomegranate and Ginger in ratio 88:10:1 to improve its flavor and nutritional quality [8].

MATERIAL AND METHODS

Procurement of material

The probiotic cultures, *Lactobacillus acidophilus* (MTCC No: 10307) and *Lactobacillus delbrueckii* subsp. *lactis* (MTCC No: 911) were procured from the Institute of Microbial Technology (IMTECH), Chandigarh. These probiotic cultures have, 'Generally Regarded As Safe' (GRAS) status. The fruits used in this study were Kinnow, Pomegranate and Ginger. These were procured from the local market. The fruits were selected after manual sorting and washed in hot water, before their extraction for juices.

Maintenance of cultures

The probiotic cultures, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis were* obtained in freeze dried forms. These cultures were revived on de Man Rogosa and Sharpe (MRS) Media and Tomato Juice Yeast Extract Milk (TJA) respectively and grown for 24 h at 37°C under aerobic conditions. Both the cultures were sub cultured fortnightly on specific media.

Purity of probiotic cultures

Purity of probiotic cultures was tested on the basis of microscopic examination, colony morphology, gram staining, catalase test and carbohydrate fermentation test.

Microscopic examination

A gram stained smear of probiotic cultures was examined microscopically in order to observe their morphology and staining property to check the purity of culture used.

Colony Morphology

Pure cultures was streaked on their respective agar plate using sterile inoculating needle and incubated at 37°C for 24 h and colonies formed were observed microscopically.

Gram staining of probiotic cultures

The probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis*, used in this study were subjected to gram staining to observe their gram reaction and then observed microscopically. Bacteria that stain purple are Gram positive and those that stain pink are said to be gram negative.

Catalase test

Both the cultures from well-isolated colonies were placed on two different clean glass slides using an inoculating needle. A drop of 3 per cent hydrogen peroxide solution was added to these cultures and closely observed for the bubble formation. The formation of bubbles was compared with that in the culture of *Escherichia coli*.

Carbohydrate fermentation test

Various carbohydrates (Lactose, Glucose, Maltose and Sucrose) were incorporated in phenol red medium. The medium was transferred in test tubes, containing Durham's tubes and sterilized by autoclaving at 15 Ibs pressure (121°C) for 15 min. After sterilization, 0.1 ml of the bacterial culture was inoculated into each sugar tube separately and incubated at 37°C. The results were noted after 24 h. In positive cases, the colour of the medium changes from red to yellow, due to acid production. Gas formation is indicated by observing gas bubbles in the Durham's tubes.

Preparation of Probiotic inoculum

The two probiotic organisms i.e. *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *lactis* were inoculated into de Man Rogosa and Sharpe (MRS) broth and Tomato Juice Yeast Extract Milk broth (10 ml) separately and incubated at 37°C for two days under aerobic condition. After growth, these probiotic cells were transferred aseptically into MRS broth and TJB broth (50 ml), respectively and incubated at 37°C for two days under aerobic condition for growth. Then cells were harvested by centrifuging at 4,000 rpm for 30 min at 4ºC from the respective broth, *L. acidophilus* and *L. delbrueckii* subsp. *lactis* cells thus obtained were again transferred to MRS broth and TJB (50 ml), respectively and incubated at 37ºC for 18 h to allow the cells to reach 10^{12}-10^{13} CFU/ml. The probiotic cells were again harvested by centrifugation at 4,000 rpm for 30 min at 4ºC. Before the probiotic cells were added to fruit juices, they were washed with sterile Phosphate Buffered Saline (PBS, pH 7.0).

Extraction of juice

Kinnow, Pomegranate and Ginger were washed with clean running water to remove dust particles and to reduce the surface microbial load. Peeled Kinnows were put in the 'Kalsi juicer machine', to squeeze out the juice. The juice was collected in the container placed below, while the pulp and seeds were removed separately. Pomegranate fruits were cut into pieces and arils were separated. Ginger was thoroughly washed, its bark like outer covering was peeled off and cut into small pieces. The recovery of Kinnow juice was 50 %, while that of Pomegranate and Ginger was 40 % and 12%, respectively. Kinnow, Pomegranate and Ginger juice were mixed in the ratio of 89:10:1.

Development of probiotic blended Kinnow juice

Blended Kinnow juice was taken in Erlenmeyer flask (500 ml capacity) and the mouth of the flask was plugged, using a cotton plug. The juice was pasteurized at 90ºC for 1min [9]. It was inoculated with probiotic cultures (10%), *L. acidophilus* and *L. delbrueckii* subsp. *lactis*, individually as well as in combination under aseptic conditions. The blended Kinnow juice was pasteurized again at 76^0C for 30s [9] and stored at 4^0C for seven weeks of storage. After every week sample was taken aseptically from probiotic juice and assessed for their microbiological and physiochemical parameters.

Analysis of the developed juices

Probiotic blended Kinnow juice was taken for analysis on 0 day and the stored product until seven weeks of storage.

Physicochemical analysis of the developed juices

pH was measured using the pH digital analyser. Total Soluble Solids (TSS) and titrable acidity were estimated by methods of AOAC [10]. Total sugars and Vitamin C content were estimated using method given by Lane and Eynon [11], described by Ranganna [12].

Microbiological analysis of the developed probiotic juices

All the developed probiotic juices were analyzed on 0 day and then after every week of storage till seven weeks. At each sampling day, 10 ml of the sample was collected aseptically and blended with 90ml of 0.1% sterile peptone water and subject and to serial dilutions.

Sensory evaluation of the developed probiotic blended Kinnow juice

The developed probiotic blended Kinnow juice was sensorally evaluated by five semi-trained panel of judges on a 9 point hedonic scale [13]. The parameters evaluated were Taste, Appearance, Color, Texture, Aroma, Mouth feel and Overall acceptability.

RESULTS

Morphology of probiotic cultures

The probiotic cultures *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis* were grown on MRS and Tomato Juice Yeast Agar media respectively. The colonies of *L. acidophilus* were moderate in size and creamish white in colour, raised, having entire margin, slimy texture and convex elevation, whereas, colonies of *L. delbrueckii* subsp. *lactis* were off-white, raised and rough.

Gram staining of probiotic cultures

The probiotic cultures used in this study were subjected to Gram Staining to observe their Gram reaction. On

microscopic examination the Gram staining of the probiotic cells revealed Gram positive cells of *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis*.

Catalase test of probiotic cultures

The catalase test of both the probiotic cultures, *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis* was performed. There was no bubble formation observed, indicating that both the probiotic cultures are catalase negative and could not mediate the decomposition of H_2O_2 to produce O_2.

Carbohydrate fermentation test

The carbohydrate fermentation test revealed that *Lactobacillus acidophilus* and *Lactobacillus delbrueckii* subsp. *lactis* fermented the sugars (Lactose, maltose, sucrose and glucose), resulting in acid production, which change the colour from red to yellow.

Probiotic viability in probiotic blended Kinnow juice

Probiotic Kinnow juice blended with Pomegranate and Ginger was prepared by adding probiotic cultures of *Lactobacillus acidophilus* and *L. delbrueckii* subsp. *lactis* individually as well as in combination and stored for seven weeks under refrigerated storage. The probiotic viability of the developed probiotic blended Kinnow juice was analysed over the entire storage period of seven weeks at 4°C. All the developed probiotic juices had a viable count of more than 10^6 CFU/ml for four weeks of storage (Table 1). A standardized probiotic food must contain a minimum amount of 10^6 CFU/ml active and live microorganisms (probiotic) at the time of consumption (Mahmoudi 2013). At the end of seven weeks of refrigerated storage, the difference in the probiotic count was found to be statistically non significant ($p<0.05$) in all the developed probiotic blended Kinnow juices. The probiotic count decreased after four weeks of storage, in blended juice containing *L. delbrueckii* subsp. *lactis* and in combination with *L. acidophilus*. Probiotic blended Kinnow juice with *L. acidophilus*, in the therapeutic dose, had a shelf life of five weeks under refrigerated conditions. There was a significant ($p<0.05$) decrease in the probiotic viable count, in the later stage of refrigerated storage.

Determination of microbial contamination in probiotic blended Kinnow juice

Enumeration of total plate count of various sets of probiotic blended Kinnow juice was carried out throughout the storage period of seven weeks. There was no count detected in probiotic blended Kinnow juices till five week of storage (Table 2). However, after fifth week, a significant increase in the total plate count was observed in all the juices. In the first five weeks of storage of the probiotic blended Kinnow juice, Yeasts and Moulds were not detected (Table 3). After five weeks, there was a significant growth of Yeasts and Moulds in all the sets of probiotic blended Kinnow juices.

The developed probiotic blended Kinnow juice was regularly analyzed for the presence of *Escherichia coli* and Coliforms to ensure the safety of the juices. Coliform and *E. coli* were not detected throughout the seven weeks of storage of the developed probiotic juice. This indicates the good quality of the developed probiotic blended Kinnow juices and assures that they are safe for consumption.

Physiochemical analysis of probiotic blended Kinnow juice

Study of pH in probiotic blended Kinnow juice

The pH of the developed probiotic blended Kinnow juice was analyzed over the refrigerated storage of seven weeks. There was a slight decrease in pH (although statistically non significant, $p< 0.05$), during the entire storage period (Table 4).

Total Soluble Solids (TSS) of probiotic blended Kinnow juice

The difference in the total soluble solids of the developed probiotic juices was monitored for seven weeks at regular intervals. After four weeks of storage, a decline in TSS content was observed (Table 5). It was found that, the difference in TSS of different sets of probiotic juices was statistically non significant ($p< 0.05$) throughout the seven weeks.

Titrable acidity of probiotic blended Kinnow juice

The titrable acidity of the developed probiotic blended Kinnow juice was monitored for seven weeks under refrigerated storage. The titrable acidity of the probiotic juices increased gradually due to production of acid (Table 6). However the increase was statistically non significant ($p< 0.05$) in different sets of probiotic blended Kinnow juice.

Total sugars in probiotic blended Kinnow juice

The difference in total sugars of the developed probiotic juices was monitored for a refrigerated storage of seven weeks. There was decrease in total sugars of all the developed probiotic juices (Table 7). Initial total sugar content of the developed juice was 6.52%, which decreased to 6.47% (*L. acidophilus*), 6.43% (*L. delbrueckii* subsp. *lactis*) and 6.45% (*L.acidophilus* and *L. delbrueckii* subsp. *lactis*) after five weeks. The difference found in the total sugars of the developed probiotic blended Kinnow juice was found to be statistically non significant ($p< 0.05$) throughout the storage period.

Vitamin C content in probiotic blended Kinnow juice

The estimation of vitamin C content of the developed probiotic juices was monitored for a refrigerated storage of seven weeks. The vitamin C content of different combination of probiotic cultures, was found to decrease throughout the storage of seven weeks (Table 8). The initial ascorbic acid content was 23.8 mg/100 ml which decreased to 20.4 mg/100ml (*L. acidophilus)*, 19.2 mg/100ml (*L. delbrueckii* subsp. *lactis*) and 19.3 mg/100ml (*L. acidophilus* and *L. delbrueckii* subsp. *lactis).* This decline in the content of vitamin C was found to be statistically significant ($p< 0.05$) for all the sets of probiotic blended Kinnow juice. However, there was statistically non significant ($p< 0.05$) difference between the vitamin C content of control and blended Kinnow juice inoculated with *L. acidophilus*.

Sensory evaluation of probiotic blended Kinnow juice

All the developed probiotic blended fruit juice was subjected to sensory analysis by a panel of five semi trained judges on the basis of nine point hedonic scale. Overall acceptability was highest for blended Kinnow juice, containing *L. acidophilus* individually, with a mean score of 8.3, which was statistically ($p< 0.05$) better than juice containing *L. delbrueckii* subsp. *lactis* and in combination of two probiotic bacteria (Table 9).

Statistical analysis showed a significant difference in taste (significant at 1% level of significance, $p< 0.01$) and aroma (significant at 5% level of significance, $p< 0.05$), whereas, other parameters (appearance, texture, colour and mouthfeel) were recorded statistically non significant.

DISCUSSION

Probiotic viability in blended Kinnow juice

The decline in viability of the probiotic count may be attributed to the decrease in pH, increase in titrable acidity and reduction in sugar content. Shah *et al* [14] examined the survival of probiotic bacteria in model fruit juice. Three different strains of probiotic bacteria were used in this study, *Lactobacillus rhamnosus* HN001, *Bifidobacterium lactis* HN001 and *Lactobacillus paracasei* LPC 37. The probiotic bacteria were inoculated into model juice with various vitamins and antioxidants, namely white grape seed extract, green tea extract, vitamin B_2, vitamin B_3, vitamin B_6, vitamin C and vitamin E. The model juice without any additives was used as a control. Their viability was assessed on a weekly basis using plate count method. The model juice was made with sucrose, sodium citrate, citric acid powder and distilled water and was pasteurized before use. Their findings showed that probiotic bacteria did not survive well in the harsh environment of the model fruit juice. However, the model juice containing vitamin C, grape extract and green tea extract showed better survival of probiotic bacteria. The model juice containing grape seed extract, green tea extract and vitamin C had the same initial population of 8.32 log CFU/ml and at the end of the 6 week storage period, it had an average viability of 4.29 log CFU/ml, 7.41 log CFU/ml and 6.44 log CFU/ml, respectively. Juices containing all other ingredients tested had viable counts of $<10^6$ CFU/ml at the end of the six week storage. Mousavi *et al* [15], developed probiotic pomegranate juice using four strains of Lactic acid bacteria: *L. plantarum, L. delbrueckii, L. paracasei* and *L. acidophilus*. *Lactobacillus plantarum* and *L. delbrueckii* showed higher viability during the storage time. Viable cells remained at their maximum level within two weeks, but decreased dramatically after four weeks. In another study, conducted by Pereira *et al* [16], probiotic cashew apple juice was prepared with an initial probiotic count of 7.48 log CFU/ml and they observed that there was a non significant decrease after 42 days of storage at 4^0C.

Study of pH in probiotic blended Kinnow juice

The decrease in pH may be due to utilization of carbohydrate by probiotic bacteria and production of small amounts of organic acids, causing lowering of pH of the fruit juices [14]. According to Ding and Shah [17], many of the free bacteria are not viable at the later stage of storage, although the dead probiotic cells could release enzymes for hydrolyzing sugar in the fruit, thus lowering the pH from 2.81 to 2.57 in probiotic orange juice, after six weeks of storage.

Yoon *et al* [18] found that there was a slow decrease in pH of beet juice prepared with probiotic lactic acid bacteria. The pH decreased from 6.3 to 5.0 after four weeks of storage at 4^0C. The decrease in pH was due to acid production by lactic acid bacteria. In another study conducted by Yoon *et al* [19], a similar trend of decline in pH of probiotic tomato juice was reported owing to the presence of lactic acid bacteria. Krasaekoopt *et al* [20] also reported a decrease in pH of probiotic fruit juices, stored at 4°C for four weeks. The pH decreased from 3.31 to 3.30 in grape juice, 3.7 to 3.6 in pineapple juice, 3.5 to 3.4 in apple juice and in red orange juice it decreased from 3.4 to 3.3.

TSS of blended Kinnow juice

The decline in TSS may be attributed to the utilization of the sugars by the lactic acid bacteria [21]. A similar report regarding decline in TSS of probiotic orange and apple juice containing eight different probiotic bacteria was given by Ding and Shah [17]. They found that TSS decreased from 11.8 °brix to 10.6 °brix after six weeks of storage. Shah *et al* [14] also reported a decrease in TSS in model juice prepared with three different probiotic strains. They noted that TSS decreased from 11.8 °brix to 9 °brix, after six weeks of storage in juice prepared with *L. rhamnosus* and *L. paracasei* and TSS decreased from 11.8 °brix to 9 °brix in juice prepared with *Bifidobacterium lactis*. In the study of Kumar *et al* [22] on physiochemical analysis of fresh and probiotic fruit juices with *Lactobacillus casei*, a similar decline in the TSS of juices was observed for an entire period of 72 h.

Titrable acidity of probiotic blended Kinnow juice

The reason for increase in titrable acidity, maybe due to production of acid, after utilization of sugar by lactic acid bacteria [14]. Khan *et al* [23] reported that acidity of plain carrot juice was 0.16%, whereas the acidity of carrot juice blends with other fruit juices ranged from 0.29-0.39%. Moraru *et al* [24] also reported an increase in acidity with the utilization of sugars in probiotic vegetables juices. Bhardwaj and Mukherjee [25] reported a minimum increase in the acidity during six months of storage, when juice was blended with kinnow, amla and ginger juice. Mohammad *et al* [26] reported an increase in the titrable acidity of the probiotic juice products of apple and orange containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum.*

Total sugars in probiotic blended Kinnow juice

Yoon *et al* [6] reported that total sugar content of cabbage juice decreased from 45.6 mg/ml to 36.5 mg/ml after 72 h of fermentation by *Lactobacillus casei*. Similarly, Moraru *et al* [24] reported decline in sugar content, in fermented vegetable juices, using *Bifidobacterium* strain BB12. It was observed that initial fermentative sugar content in beetroot was 45 mg/100g which decreased to 25 mg/100g after 72 h of fermentation.

Vitamin C content in probiotic blended Kinnow juice

The ascorbic acid content of the juice decreased during storage, which was probably due to the fact that ascorbic acid, being sensitive to oxygen, light and heat was oxidized easily in the presence of oxygen by both enzymatic and non-enzymatic catalyst. Tripathi *et al* [27] reported a continuous decrease in ascorbic acid content from 17.0-19.0 mg/100gm in all the blends of pineapple: guava RTS beverage during three months of storage. Bhardwaj and Mukherjee [25] reported different blends of probiotic Kinnow juice. The content of vitamin C decreased from 18.67 to 1.08 mg/100ml after six months of storage. Baljeet *et al* [28] reported a significant decrease in ascorbic acid content from 1.43 to 1.20 mg/100ml in Whey based pineapple and bottle guard mixed herbal beverage.

CONCLUSION

From the results of this study, it is concluded that out of two probiotic cultures (*L. acidophilus* and *L. delbrueckii* subsp. *lactis*), *L. acidophilus* is a better probiotic as compared to *L. delbrueckii* subsp. *lactis*. Alongwith this, it was also concluded that blended fruit juice could be used for probiotication by lactic acid bacteria and the product could serve as a health beverage for consumers who are allergic to dairy products.

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29. Preservation of Food Adulteration Rules (1956) Appendix D Table 2 Microbiological requirement of food products. Bureau of Indian Standards, New Delhi, India.

Table 1: Probiotic count (log CFU/ml) in probiotic blended Kinnow juice

Probiotics	Weeks								Mean ± SE
	0	1	2	3	4	5	6	7	
LA	12.4	9.5	8.7	7.6	6.8	6.1	5.8	5.4	$7.900^{a} \pm 0.798$
LD	12.1	9.1	8.1	6.4	6.0	5.8	5.6	4.8	$7.238^{a} \pm 0.853$
LA+LD	12.2	9.3	8.5	7.0	6.1	5.7	5.2	4.9	$7.363^{a} \pm 0.883$
Control	-	-	-	-	-	-	-	-	-

Values followed with different superscript are significantly different ($p<0.05$)

Table 2: Total plate count (log CFU/ml) of probiotic blended Kinnow juice

Probiotics	Total plate count (log CFU/ml)		Microbiological Specifications*
	Weeks		
	6	7	
LA	0.578	1.010	<50 CFU/ml
LD	0.825	1.611	
LA+LD	0.776	1.501	
Control	0.667	1.323	

* PFA [29]

Table 3: Enumeration of Yeast and Mould count (logCFU/ml) in probiotic blended Kinnow juice

Probiotics	Yeast and Mould count (logCFU/ml)		Microbiological Specifications*
	Weeks		
	6	7	
LA	0.278	0.331	<2.0 CFU/ml
LD	0.591	0.798	
LA+LD	0.334	0.632	
Control	0.451	0.593	

* PFA [29]

Table 4: Change in pH of probiotic blended Kinnow juice

Probiotics	Weeks								Mean ± SE
	0	1	2	3	4	5	6	7	
LA	3.56	3.56	3.56	3.54	3.53	3.50	3.49	3.48	$3.5275^{ab} \pm 0.01176$
LD	3.56	3.56	3.55	3.51	3.50	3.47	3.45	3.39	$3.4988^{b} \pm 0.02125$
LA+LD	3.56	3.56	3.56	3.55	3.54	3.50	3.49	3.46	$3.5275^{ab} \pm 0.01373$
Control	3.56	3.56	3.56	3.56	3.56	3.55	3.54	3.51	$3.5500^{a} \pm 0.00627$

Values followed with different superscript are significantly different ($p< 0.05$)

Table 5: Change in TSS (°brix) of probiotic blended Kinnow juice

Probiotics	Weeks								Mean ± SE
	0	1	2	3	4	5	6	7	
LA	15.6	15.6	15.5	15.4	15.4	15.0	14.6	14.3	$15.163^{a} \pm 0.1721$
LD	15.6	15.5	15.4	15.2	14.8	14.6	14.2	14.0	$14.913^{a} \pm 0.2150$
LA+LD	15.6	15.6	15.5	15.4	15.2	15.1	14.5	14.2	$15.138^{a} \pm 0.1851$
Control	15.6	15.6	15.6	15.4	15.3	15.2	15.1	14.9	$15.338^{a} \pm 0.0925$

Values followed with different superscript are significantly different ($p< 0.05$)

Table 6: Titrable acidity (%) in probiotic blended Kinnow juice

Probiotics	Weeks								Mean ± SE
	0	1	2	3	4	5	6	7	
LA	0.76	0.77	0.78	0.80	0.82	0.83	0.84	0.85	$0.8062^{a} \pm 0.01194$
LD	0.76	0.76	0.77	0.78	0.80	0.81	0.82	0.83	$0.7913^{a} \pm 0.00972$
LA+LD	0.76	0.77	0.78	0.80	0.81	0.82	0.82	0.84	$0.8013^{a} \pm 0.01025$
Control	0.76	0.76	0.76	0.77	0.78	0.79	0.81	0.81	$0.7800^{a} \pm 0.00756$

Values followed with different superscript are significantly different ($p< 0.05$)

Table 7: Total sugars (%) of probiotic blended Kinnow juice

Probiotics	Weeks								Mean ± SE
	0	1	2	3	4	5	6	7	
LA	6.52	6.52	6.52	6.50	6.49	6.47	6.43	6.42	$6.4625^{a} \pm 0.01426$
LD	6.52	6.52	6.51	6.48	6.46	6.43	6.40	6.38	$6.4713^{a} \pm 0.01934$
LA+LD	6.52	6.52	6.51	6.49	6.47	6.45	6.41	6.40	$6.4838^{a} \pm 0.01684$
Control	6.52	6.52	6.52	6.51	6.49	6.48	6.46	6.43	$6.4913^{a} \pm 0.01172$

Values followed with different superscript are significantly different ($p< 0.05$)

Table 8: Vitamin C (mg/100ml) in probiotic blended Kinnow juice

Probiotics	Weeks								Mean ± SE
	0	1	2	3	4	5	6	7	
LA	23.8	23.5	23.1	22.8	22.3	21.0	21.2	20.4	$22.263^{a} \pm 0.445$
LD	23.8	23.0	22.5	21.3	21.2	20.1	19.7	19.2	$21.350^{ab} \pm 1.466$
LA+LD	23.8	23.2	22.4	21.7	21.4	20.7	19.9	19.3	$21.550^{ab} \pm 0.987$
Control	23.8	23.8	23.8	22.4	22.1	21.8	20.2	19.3	$22.150^{a} \pm 0.602$

Values followed with different superscript are significantly different ($p<0.05$)

Table 9: Sensory evaluation of probiotic blended Kinnow juice

Probiotics	Parameters						
	Taste	Appearance	Colour	Texture	Aroma	Mouthfeel	Overall Acceptability
LA	8.6	8.4	8.3	7.6	8.2	8.3	8.3
LD	8.2	8.3	8.2	7.4	7.9	7.9	7.9
LA+LD	8.4	8.4	8.3	7.5	8.0	8.0	8.1
Control	8.9	8.4	8.3	7.6	8.6	8.4	8.4
$\chi 2$ value	93.936^{**}	0.110^{NS}	8.002^{NS}	1.842^{NS}	3.981^{*}	0.883^{NS}	9.023^{*}

The developed probiotic juices were sensorally evaluated by semi-trained panel of judges on a 9 point hedonic scale [13].

***significant at 5% level of significance ($p< 0.05$)

** significant at 1% level of significance ($p< 0.01$)

NS – non significant