Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 638-650

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 638-650**
Journal homepage: http://www.ijcmas.com

Original Research Article

Investigation of Microbial, Physicochemical and Color Properties of Probiotic Pomegranate Beverage during Storage

Mamta Thakur[1*], H. W. Deshpande[2] and M. A. Bhate[3]

[1]Department of Food Trade and Business Management, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani - 431 402 (Maharashtra) India
[2]Department of Food and Industrial Microbiology, College of Food Technology, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani - 431 402 (Maharashtra) India
[3]Department of Microbiology, Shivaji College, Parbhani- 431 402 (Maharashtra) India
**Corresponding author*

A B S T R A C T

The aim of present work is to investigate the microbial, physico-chemical and color attributes of probiotic pomegranate beverage kept at room temperature (20°C) and refrigeration temperature (4°C) during 28 days of storage. The microbial analysis was carried out by standard plate count method. The physico-chemical properties were determined using their standard procedure and color was estimated using Color HunterLab. The viability of probiotic cultures was lost completely in beverage stored at 20°C but it was $2.5x10^7$ CFU/mL at 4°C. Yeast and mold count was $1.9x10^8$ CFU/mL in former beverage at the end, while no traces were found in latter. No coli-form colonies were detected in both cases. Regarding chemical properties, TSS, titratable acidity, pH, glucose, fructose, total sugars, reducing sugars, non-reducing sugars, ascorbic acid, phenol and tannin content for beverage stored at 20°C was 7.6°Bx, 1.17%, 3.263, 52 g/L, 58.46 g/L, 10.53%, 10.41%, 0.12%, 7.29 mg/100mg, 202.32 mg/100g and 0.091%, respectively during first week of storage while for other beverage, values were 12.2°Bx, 0.987%, 3.281, 55.47 g/L, 59.64 g/L, 10.07%, 9.93%, 0.14%, 7.32 mg/100g, 202.71 mg/100g and 0.094%, respectively for 4th week. Further, L* and a* value and h° decreased while b* and c* value increased during storage which was higher for room temperature stored beverage indicating the immense yellow intensity in beverage. Hence, it can be concluded that refrigerated probiotic beverage was found to be more stable regarding microbial, physico-chemical and color attributes during storage whereas beverage kept at room temperature could be microbiologically safe for one week.

Keywords

Ambient Storage, Physico-Chemical Characteristics, Probiotic Pomegranate Beverage, Refrigerated Storage, Viability

Introduction

Food industry has high expectations with food products that meet the consumers' demand for a healthy life style. In this concern, 'probiotic foods' play a significant role. Probiotics are defined as live micro-organisms, which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). The health benefits include boosting the immune system, reducing rotavirus diarrhoea, alleviating symptoms of lactose intolerance, decreasing faecal bacterial enzyme activity and mutagenicity, preventing the recurrence of superficial bladder cancer and atopic

diseases (Kalliomaki *et al.*, 2001). For receiving health benefits, the viable cell count of probiotics should be 10^7-10^9 CFU/ml in the product (Shah, 2001).

Fruit juices and beverages are considered as ideal in the issues of lactose intolerance, vegan lifestyles and the demand for low-fat and low-cholesterol (Ranadheera *et al.*, 2010). Pomegranate juice, in addition to being delicious and refreshing, possesses many health benefits as it is rich in polyphenols such as gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, o-and p-coumaric acids, catechin and quercetin as well as enzymes, proteins, pectins and anthocyanins (Du *et al.*, 1975).

Numerous studies have been conducted to demonstrate the potential of growth of probiotic strains in fruit juices like tomato, beet and cabbage juices and showed that all the strains are capable of growth in the fruit juices mentioned (Yoon *et al.*, 2004, 2005, 2006). In the case of probiotic products, storage temperature has substantial impact on maintaining product integrity. Generally probiotic based products must be kept under refrigerated storage (Mohammadi and Mortazavian, 2011). However, probiotic product might be subjected to cold chain interruption for hours/days during industrial distribution, retailing and domestic usage. This leads to the question whether probiotic bacteria declines during ambient storage to such an extent that there are not enough bacteria remaining in the product to be useful. And whether there is desired level of chemical attributes in product. The answer of this question for fruit based probiotic product was not found in literature.

Hence, the aim of present study was to compare the viability of cultures in probiotic pomegranate beverage stored at ambient (20^oC) and refrigeration (4^oC) temperature and to evaluate the consumption of various substrates in beverage thus observing the metabolism of probiotic cultures to examine the possibility of producing a probiotic pomegranate beverage with improved health benefits.

Materials and Methods

Preparation of pomegranate juice

Freshly harvested pomegranate fruits (cv. *Bhagwa*) were procured from local market of Parbhani (Maharashtra) India. Pomegranate juice was prepared by blending the juicy arils in the domestic mixer. Its total soluble solids was fixed to 13^oBx and stored at 4^oC before use.

Probiotic strains and culture

Lactobacillus isolates, *Lactobacillus bulgaricus and L. plantarum*, were isolated and identified using phenotypic and genotypic methods in Department of Food and Industrial Microbiology, College of Food Technology, VNMKV, Parbhani and Department of Microbiology, Shivaji College, Parbhani (India) along with Agharkar Research Institute, Pune (India).

L. plantarum and *L. bulgaricus* was cultivated separately in the MRS broth for 24 h at 37^oC. To obtain the biomass, 10 mL of the separately cultivated MRS broths were mixed in equal proportion (1:1) and centrifuged at 4000 rpm for 10 min. The obtained biomass was washed and thus, inoculum was prepared (Mousavi *et al.*, 2010). It was then introduced into pasteurized pomegranate juice (100 mL) for making 10% concentration of probiotics. The inoculated juice was then incubated at 37^oC for 24 h and was treated as starter culture for preparation of final beverage.

Preparation of probiotic pomegranate beverage

Above prepared starter culture (10 mL) was then added to the pasteurized pomegranate juice (100 mL) to obtain 10% inoculation. It was allowed to ferment at 37^{o}C for 7 h. After this, it was kept at ambient (20^{o}C, sample A) and refrigeration temperature (4^{o}C, sample B) for 4 weeks packed in glass bottles. The microbiological and chemical properties were assessed on the 0, 7, 14, 21 and 28 days of ambient and refrigerated storage.

Microbial analysis of probiotic pomegranate beverage

The viable count of mixed culture was determined by the standard plate count method using Man-Rogosa-Sharpe agar (MRS agar) on the 0, 7, 14, 21 and 28 days and the results were expressed as CFU ml^{-1} beverage.

The yeast and mold count of beverage was determined using potato dextrose agar medium. The coli-form and basically *E. coli* are the indicator microbes of water contamination by feces. The coli-form gives red pink color colonies on the MacConkey agar. Plates were incubated at 37^{o}C for 48-72 hours (Chris, 2006).

Chemical analysis of probiotic pomegranate beverage during storage

Total soluble solids (T.S.S.), Titratable acidity and pH

Total soluble solids were measured using hand refractometer (ERMA make). Titratable acidity, expressed as per cent lactic acid, was determined by titration against 0.1N NaOH using phenolphthalein as an end point indicator. The pH value was obtained by using a digital pH meter (ELICO LI612) after standardizing it with buffers of pH 4.0 and 9.0 (Ranganna, 1991).

Glucose and fructose

The glucose and fructose content were determined in beverage by phenol sulfuric acid method (Nielsen, 2010).

Total Sugars, Reducing Sugars and Non-reducing sugars

Total carbohydrate/sugars was estimated by standard procedure using phenol sulphuric acid (Nielsen, 2010).

The amount of reducing sugar of beverage was calculated by Nelson – Somogyi method (Syed *et al.*, 2007) and non-reducing sugars was obtained by subtracting reducing sugars from total sugars.

Ascorbic acid (vitamin C)

Ascorbic acid contents of samples were determined according to the titration method using 2, 6-dichlorophenol indophenols (Ranganna, 1991).

Total phenolic content

The concentration of phenolic compounds was determined by the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) where 5g of sample was homogenized in 25 mL of 50% (v/v) ethanol/water solution. The sample (100 µL) was mixed with 5 mL of the 0.2N Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate. The mixture was kept for 2 h at room temperature in the dark before the absorbance was measured at 765 nm spectrophotometrically. The total phenolic content was expressed as mg gallic acid equivalents (mg GAE/100 mL).

Tannin content

Total tannin content of sample was measured by Folin Denis method (Saxena *et al.,* 2013) which is based on the measurement of blue color formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution.

Color

Color HunterLab L*a*b* (Hunterlab ColorFlex EZ) was calibrated using a white and black standard ceramic tile. The parameter a* takes positive values for reddish colours and negative values for the greenish ones, whereas b* takes positive values for yellowish colours and negative values for the bluish ones.

L* is an approximate measurement of luminosity. Chroma (c*) is considered as the quantitative attribute of colourfulness. Hue angle (h*) defines the difference of a certain colour with reference to grey colour with the same lightness (Weaver and Daniel, 2005). Numerical values of L*, a* and b* were converted into ΔE (total colour difference) according to Eq. (1).

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2} \quad (1)$$

Statistical analysis

All experiments were run in triplicate. Analysis of variance was calculated using standard ANOVA procedure. The data obtained for various treatments was recorded and statistically analyzed by factorial complete randomized design (FCRD) to find out the level of significance as per the method proposed by Panse and Sukhatme (1957). The analysis of variance revealed at significance at $P< 0.05$ level. The standard error (SE) and critical difference (CD) at 5 % level were mentioned where required.

Results and Discussion

Effect of storage on viability of probiotics

The changes in the viability of probiotic microorganisms during the storage are tabulated in Table 1. It is observed that the probiotic cultures were capable of surviving in the product at 4^oC for 28 days. The initial microbial population was same (6.5×10^9 CFU/mL) in both samples just after fermentation. Over the storage period, viable count reduced in both cases. However, reduction was very sharp for sample A stored at ambient temperature. At the end of 28 days, the microbial count reduced to 2.5×10^7 CFU/mL for sample B and no viability was detected in sample A stored at room temperature (20^oC). This may be due to the extremely high metabolic activity of microbes at room temperature leading to accumulation of large quantity of lactic acid and other metabolic products. *L. delbrueckii* ssp. *bulgaricus* produces a large amount of acid (sharp acidification), hydrogen peroxide and possibly bacteriocins resulting in the suppression of probiotic microorganisms (Sarvari *et al.,* 2014).

Temperature increases the mortality effect of organic acids and is most critical factor on the viability of probiotics. The cell wall of lactic acid bacteria consists of saturated, unsaturated and cyclic carbon chains, which will vary depending on parameters like temperature, pH, NaCl concentration and medium content. Linoleic and oleic synthesis will occur at acidic situations. These acids will absorb hydrogen in an acidic environment increasing the permeability of proton in membranes, and therefore, leading to viability increase when confronted with hostile conditions

throughout acidic situation during storage at refrigeration temperature (Sheehan *et al.*, 2007).

The results obtained for room temperature viability of microbes can be explained with those found by Ferdousi *et al.*, (2013) who said that considerable loss in viability of probiotics in room temperature could be attributed to increasing cell metabolism and death at higher temperatures as compared to refrigerated storage.

In the present investigation, the microbial count was detected higher than 10^6 CFU/mL for sample B. The present results for sample B were better than those obtained by Mousavi *et al.*, (2010) for probiotic pomegranate juice. High survival rates of *L. casei* in non-dairy fermented products stored refrigerated was reported by Pereira *et al.*, (2011) who studied *L. casei* fermentation and survival in cashew apple juice.

Effect of storage on microbiological properties of probiotic beverage

The yeast and mold count and coli-form count were detected in both the samples over the storage period and presented in Table 2. In the present work, yeast and mold count was observed in the sample A stored at room temperature and in probiotics based products; the growth of microbes other than the added culture is considered to be undesirable. During the second week, the probiotic culture count reduced significantly due to the stressful conditions of low pH in sample A due to lactic acid accumulation.

This low pH environment is best suited for the growth of yeasts and mold. The count observed was $8.5x10^2$ CFU/mL for sample A at the end of 14^{th} day and it further increased upto $1.9x10^8$ CFU/mL. On the other hand, there was no yeast/mold colonies detected in sample B. This may be due to low temperature of storage and thus less metabolic activity.

On the other hand, no colony of coli-form bacteria was detected in both the samples over the entire storage period showing the hygienic conditions of processing of product because the water used for analysis work was double distilled.

Effect of storage on chemical properties of probiotic beverage

Total soluble solids, Titratable acidity and pH

It can be observed from Table 3 that initially, the probiotic pomegranate beverage had 13 ^oBx total soluble solids (TSS). During the storage period, the value of total soluble solids of sample B dropped from 13 to 12.2 ^oBx, while this drop was significantly higher in case of sample A where TSS reduced to 7.6 ^oBx from 13 ^oBx within 7 days. For next days of storage period, the sample A was not considered because it was not safe for consumption due to yeast and mold growth at ambient temperature. The decrease of TSS was significantly lower in sample B than sample A due to higher temperature of storage resulting in high metabolic activity in case of sample A.

Regarding the titratable acidity and pH, they showed reverse relation respective to each other. The titratable acidity expressed in lactic acid significantly increased over the storage period while pH lowered. After incubation, 0.54 percent titratable acidity was found in the probiotic pomegranate beverage. It increased significantly to 1.17 per cent of lactic acid within 7 days. On the other hand, increase in acidity was slightly less rapid in sample B stored at refrigeration temperature.

Table.1 Effect of storage during 4 weeks on viability of probiotic cultures in probiotic pomegranate beverage

S. No.	Samples	Storage Period				
		0 Day	7 Days	14 Days	21 Days	28 Days
1	A	6.5×10^9	2.3×10^7	5.3×10^4	1.2×10^1	ND
2	B	6.5×10^9	1.4×10^9	5.3×10^8	9.3×10^7	2.5×10^7
ANOVA						
				F-value	SE±	CD at 5 %
		T		282	0.0791	0.2331
		D		352.37	0.1251	0.3686
		T x D		219.77	0.1770	0.5213

Each value is an average of three determinations
ND - not detected; T- Treatment; D – Storage days

Table.2 Microbiological analysis of probiotic pomegranate beverage during storage period

S. No.	Samples	Yeast and mold count (CFU/mL)					Coli-form count (MPN/mL)				
		Storage Period (Days)					Storage Period (Days)				
		0	7	14	21	28	0	7	14	21	28
1	A	ND	ND	8.5×10^2	3.2×10^6	1.9×10^8	ND	ND	ND	ND	ND
2	B	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ANOVA											
		F-value		SE±	CD at 5 %		F-value		SE±	CD at 5 %	
	T	728.39		0.0770	0.2268		-		-	-	
	D	210.13		0.1217	0.3587		-		-	-	
	T x D	210.13		0.1722	0.5073		-		-	-	

Each value is an average of three determinations
ND – Not detected; T- Treatment; D – Storage days

Table.3 Effect of storage during 4 weeks on TSS, titratable acidity and pH in probiotic pomegranate beverage

S. No.	Samples	Total soluble solids (^oBx)					Titratable Acidity (% lactic acid)					pH				
		Storage Period (Days)					Storage Period (Days)					Storage Period (Days)				
		0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
1	A	13	7.6	NA	NA	NA	0.54	1.17	NA	NA	NA	3.512	3.263	NA	NA	NA
2	B	13	12.7	12.5	12.4	12.2	0.54	0.653	0.704	0.813	0.987	3.512	3.494	3.467	3.353	3.281
ANOVA																
		F-value		SE±	CD at 5 %		F-value		SE±	CD at 5 %		F-value		SE±	CD at 5 %	
	T	177,641.657		0.014	0.042		1858.8		0.0065	0.0191		54280		0.0006	0.0018	
	D	19,430.129		0.022	0.067		455.13		0.0103	0.0303		95031		0.0009	0.0029	
	TxD	16,046.521		0.032	0.094		946.57		0.0145	0.0429		80918		0.0041	0.0041	

Each value is an average of three determinations
NA – Not Applicable because product is not safe for consumption
T- Treatment; D – Storage days

Table.4 Effect of storage during 4 weeks on glucose and fructose profile in probiotic pomegranate beverage

S. No.	Samples	Glucose (g/L)					Fructose (g/L)				
		Storage Period (Days)					Storage Period (Days)				
		0	7	14	21	28	0	7	14	21	28
1	A	60.35	52	NA	NA	NA	62.82	58.46	NA	NA	NA
2	B	60.35	58.67	57.28	56.32	55.47	62.82	61.77	60.83	60.09	59.64
		ANOVA									
		F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %				
	T	3,240,430.780	0.014	0.041	4,399,327.104	0.012	0.037				
	D	560,769.876	0.022	0.065	771,026.479	0.020	0.058				
	T x D	445,578.022	0.031	0.092	669,716.559	0.028	0.082				

Each value is an average of three determinations
NA – Not Applicable because product is not safe for consumption
T- Treatment; D – Storage days

Table.5 Effect of storage during 4 weeks on sugars profile in probiotic pomegranate beverage

S. No.	Samples	Total sugars (percent)					Reducing sugars (percent)					Non-reducing sugars (percent)				
		Storage Period (Days)					Storage Period (Days)					Storage Period (Days)				
		0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
1	A	13.14	10.53	NA	NA	NA	12.32	10.41	NA	NA	NA	0.82	0.12	NA	NA	NA
2	B	13.14	12.19	11.33	10.61	10.07	12.32	11.58	10.93	10.36	9.93	0.82	0.61	0.40	0.25	0.14
		ANOVA														
		F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %						
	T	113,225.745	0.014	0.042	122,677.035	0.013	0.039	3,072.137	0.003	0.010						
	D	29,545.525	0.022	0.066	29,813.061	0.021	0.061	3,439.558	0.005	0.015						
	T x D	14,777.568	0.032	0.094	17,093.754	0.029	0.087	362.172	0.007	0.022						

Each value is an average of three determinations
NA – Not Applicable because product is not safe for consumption
T- Treatment; D – Storage days

Table.6 Effect of storage during 4 weeks on profile of ascorbic acid, total phenol and tannin content in probiotic pomegranate beverage

S. No.	Samples	Ascorbic acid (mg/100g)					Total phenolic content (mg/100g)					Tannin content (per cent)				
		Storage Period (Days)					Storage Period (Days)					Storage Period (Days)				
		0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
1	A	9.53	7.29	NA	NA	NA	206.64	202.32	NA	NA	NA	0.114	0.091	NA	NA	NA
2	B	9.53	8.64	8.27	7.8	7.32	206.64	205.32	204.57	203.28	202.71	0.114	0.108	0.103	0.099	0.094
		ANOVA														
		F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %						
	T	32385	0.0194	0.0572	40165	0.1369	0.4032	15,473.221	0.000	0.001						
	D	7818.7	0.0307	0.0905	68559	0.2164	0.6375	3,215.102	0.001	0.002						
	T x D	4115.4	0.0434	0.1280	65324	0.3061	0.9017	1,962.640	0.001	0.002						

Each value is an average of three determinations
NA – Not Applicable because product is not safe for consumption
T- Treatment; D – Storage days

Table.7a Effect of storage during 4 weeks on color (l*, a* and b*) pattern in probiotic pomegranate beverage

S. No.	Samples	L* value					a* value					b* value				
		Storage Period (Days)					Storage Period (Days)					Storage Period (Days)				
		0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
1	**A**	24.39	19.75	NA	NA	NA	15.14	14.27	NA	NA	NA	2.57	2.76	NA	NA	NA
2	**B**	24.39	23.21	22.96	22.23	21.69	15.14	15.03	14.86	14.59	14.32	2.57	2.61	2.65	2.69	2.74

ANOVA

	L* value			a* value			b* value		
	F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %
T	13500	0.0860	0.2533	32866	0.0109	0.0323	89,805.624	0.004	0.011
D	2293.7	0.1360	0.4006	57571	0.0173	0.0511	14,111.517	0.006	0.018
T x D	1757.2	0.1923	0.5665	50292	0.0245	0.0723	16,444.027	0.008	0.025

Each value is an average of three determinations
NA – Not Applicable because product is not safe for consumption
T- Treatment; D – Storage days

Table.7b Effect of storage during 4 weeks on color (c* and h*) pattern in probiotic pomegranate beverage

S. No.	Samples	c* value					h* value				
		Storage Period (Days)					Storage Period (Days)				
		0	7	14	21	28	0	7	14	21	28
1	**A**	19.5	21.66	NA	NA	NA	31.13	28.35	NA	NA	NA
2	**B**	19.5	20.12	20.64	20.89	21.03	31.13	30.62	29.79	28.98	28.16

ANOVA

	c* value			h* value		
	F-value	SE±	CD at 5 %	F-value	SE±	CD at 5 %
T	11283	0.0081	0.0239	1,881,406.716	0.009	0.027
D	17498	0.0128	0.0378	357,027.784	0.015	0.043
T x D	21301	0.0181	0.0535	276,092.281	0.021	0.061

Each value is an average of three determinations
NA – Not Applicable because product is not safe for consumption
T- Treatment; D – Storage days

At the end of 28 days, the value of titratable acidity reached to 0.987 per cent, which was even less than the value obtained at 7th day by sample A.

The results of data in Table 3 showed that the pH value was found to be 3.512 in both samples at the very initial day of storage analysis. Later on, the drop of pH value was significantly lower in sample B due to slow metabolic activity of culture at refrigeration temperature than sample A. However, the probiotic cultures reduced the pH of product from an initial value of 3.512 to 3.263 in sample A after 7 days of storage due to their ability to produce a greater amount of lactic acid. As shown in Table 3, the acidity was increased to 0.987 percent for which pH reduced to 3.281 for sample B.

The reason for decrease in total soluble solids and pH and increase in titratable acidity can be explained as probiotic microorganisms may have metabolized the simple sugars present in the juice, resulting in consequent production of small quantities of organic acids (Shah *et al.,* 2010) or the release of enzymes from dead bacteria may have hydrolyzed the juice sugars (Ding and Shah, 2008).

Similar results regarding decrease in the value of pH and increase in titratable acidity was obtained by Yoon *et al.,* (2004) in probiotication of tomato juice by *Lactobacillus plantarum* C3.

Glucose and fructose

The changes in glucose and fructose content probiotic pomegranate beverage w.r.t. storage conditions are presented in Table 4.

The data revealed that the glucose and fructose content decreased significantly during the storage of 28 days in the probiotic pomegranate beverage presenting a reduction of 60.35, 58.67, 57.28, 56.32 and 55.47g/L and 62.82, 61.77, 60.83, 60.09 and 59.64g/L, respectively for sample B stored at 4^{o}C. The sample B had significantly higher values of glucose as well as fructose than sample A in which at the end of first week, the values reduced to 52 g/L and 58.46 g/L for glucose and fructose, respectively. The possible reason for this may be that glucose and fructose were both metabolized by all strains. Similar results were obtained by Rodrigues *et al.,* (2011) who reported that the glucose and fructose levels decreased during storage, which was attributed *Lactobacillus paracasei L26* growth and sugar fermentation.

Total sugars, Reducing sugars and Non reducing sugars

The data shown in Table 5 showed that the total sugars, reducing sugars and non-reducing sugars decreased significantly for both sample A and B over storage period. In case of sample B, the total sugars, reduced sugars and non-reducing sugars varied in following range 13.14 – 10.07%, 12.32 – 9.93% and 0.82- 0.14%, respectively. In sample A, these values significantly reduced to 10.53, 10.41 and 0.12 per cent at the end of 7th day.

The reason for reduction of sugars in the both samples is the consumption of sugars by probiotic microbes for their growth. These results are found to be in line with Pakbin *et al.,* (2014) who stated that sugar was somewhat consumed by lactic acid bacteria in peach juice during probiotic fermentation. *Lactobacillus delbrueckii* consumed the greatest amount of sugar content of peach juice during fermentation when compared with other strains and decreased the initial sugar content of 20.2 g/L to 12.9 g/L.

Ascorbic acid, Total phenol content, tannin

From Table 6, it can be found that ascorbic acid content was reduced during storage. In both the samples, the initial ascorbic acid content (9.53 mg/100g) was significantly higher than sample A (7.29mg/100g; at the end of 7 days) and sample B (7.32mg/100g; at the end of 28 days). The lower ascorbic acid content in sample A was attributed to the high temperature of storage. According to Padayatty *et al.,* (2003), climate, especially temperature affects vitamin C level. Areas with cool nights produce citrus fruits with higher vitamin C levels than hot tropical areas.

The ascorbic acid content decreased significantly i.e. 9.53, 8.64, 8.27, 7.8 and 7.32 mg/100g as the storage period progressed for sample B. The ascorbic acid begins to degrade immediately after harvest and degrades steadily during prolonged storage (Murcia *et al.,* 2000). Thus, for present study, the reduction of 23.19% was observed in sample B at the end of 4 weeks which was slightly higher than that obtained by Pereira *et al.,* (2013). According to them, the reduction of ascorbic acid was less intense in the fermented cashew apple juices (19.17 and 21.49%, for probiotic cashew apple with and without sucrose addition, respectively) compared to the non-fermented sample (40.58%).

The phenolic compounds not only inhibit the growth and activity of spoilage microorganisms, but also have stimulation effect on the growth of probiotic bacteria (Shah *et al.,* 2010). A significant reduction (from 206.64 - 202.32 mg/100g) was observed in sample A for only 7 days. The phenol content varied from 206.64 to 202.71 mg/100g for probiotic beverage stored at 4^{o}C. Thus, the sample B retained significantly higher content of total phenol than sample A over the storage period. This may be attributed to low metabolism rate of microbes at refrigeration temperature than sample A. The phenolic compounds found in fresh fruit juice are generally glycosylated with sugar that on fermentation of the juice and sugar consumption by microorganism undergo deglycosylation and release of the free hydroxyl groups and relevant aglycones which can contribute to the improved functional properties of the litchi juice (Kalita *et al.,* 2015). And thus their content reduced over the storage period due to the activity of probiotics. The results related to reduction of phenolic content are similar to those found by Kalita *et al.,* (2015) for the litchi juice during fermentation period and storage period up to 4 weeks.

The tannin content of pomegranate fruit juice also contributes to its antioxidant activity. The tannin content decreased significantly for both samples during the storage. Its value ranged from 0.114 to 0.091 percent for sample A till the 7^{th} day of storage. On the contrary, the decrease in tannin content was moderate in sample B, which ranged from 0.114, 0.108, 0.103, 0.099 and 0.094% for 0, 7, 14, 21 and 28 days of storage, respectively.

Color

It can be observed from Table 7a and 7b that there was significant difference between treatments, storage days and interaction between treatments and storage for L*, a*, b*, c* and h* parameters of color. A decrease in probiotic beverage luminosity (L*) was observed in both the samples. The value of luminosity significantly reduced from initial value of 24.39 to 19.75 (till 7^{th} day) for sample A while in case of sample B, it ranged from 24.39 to 21.69 over the length of storage period. The sample A had

significantly low L* value than the other sample. This is because during storage, there was an increase in turbidity in probiotic beverage caused by lactic acid culture growth and at higher temperature as in sample A the multiplication of microbes is very fast.

Similarly, a decrease was found in a* value of both samples of probiotic pomegranate beverage which meant lowering of dark red color of product. This decrease was significantly high for sample A than sample B. The a* value ranged from 15.14 to 14.32 over the storage period for sample B. However, both the samples showed an increased value of b* color parameter during the storage period; indicating that the juices became more yellow. The initial b* value was 2.57 for both samples but at the end of 28 days, the b* value was found to be 2.74 for sample B.

The total color change ΔE* (reference value was day 0 of storage) increased in the sample A. This increase might be attributed to the increase in the yellow intensity which was significantly higher than sample B such that the human eye can perceive the color variation. Browning was not observed in the sample A. On the other hand, for sample B, total color change was less intense than sample A. Despite the instrumental color change, the human eye cannot perceive small color variations. For sample A, ΔE* value is greater than 3, thus showing a visual color change only after 7 days. But sample B did not show a perceptive color change along the storage. Thus, sample B was more stable in respect to color

The probiotic culture was likely suspended in the solution in the form of particles, which were too small to precipitate but large enough to refract light. During storage, the changes in color and turbidity caused by probiotic cultures supplementation were intensified (Pimental *et al.,* 2015). The increased turbidity and intensified color most likely resulted from the accumulation of dead or lysed bacterial cells (Shah *et al.,* 2010). The color intensification may have been caused by oxidative and non-oxidative reactions of polyphenols, resulting in colored condensation products; and to a lesser extent, by the Maillard reaction or formation of melanoidins (Tajchakavit *et al.,* 2001).

Viability was lost very sharply in beverage stored at room temperature while viability of probiotic cultures was maintained in useful range (10^9-10^7 CFU/mL) in refrigerated probiotic beverage for the 28 days. Further, yeast and mold growth was observed at room temperature after 1 week. No colony of coli-form was detected in beverage during storage period. On the other hand, the chemical constituents were consumed slowly by cultures at refrigeration conditions for 4 weeks. These findings highlighted the increased rate of metabolism of probiotic cultures at ambient conditions making it unsuitable to keep the beverage in room temperature. The yellow intensity in beverage stored at ambient temperature was significantly higher than cold stored beverage. Hence, it can be concluded that the probiotic pomegranate beverage must be stored at refrigeration temperature to possess desired level of chemical constituents and nutraceutical components but can be stored safely at ambient temperature for 7 days only.

References

Chris B, Paul N and Anthony P W 2006. Food Microbiology and Laboratory Practices. Blackwell Publishing, State Avenue, USA

Di Cagno R, Minervini G, Rizzello C G, De

Angelis M and Gobbetti M 2011. Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies *Food Microbiol* 28 1062-1071

Ding W K and Shah N P 2008. Survival of free and microencapsulated probiotic bacteria in orange and apple juices *Int Food Res J* 15 219-232

Du C T, Wang P L and Francis F J 1975. Anthocyanins of pomegranate (*Punica granatum) J Food Sci* 40 417-418

FAO/WHO 2001. Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria. Cordoba, Argentina, pp 1-4

Ferdousi R, Rouhi M, Mohammadi R, Mortazavian A M, Khosravi-Darani K and Rad A H 2013. Evaluation of probiotic survivability in yogurt exposed to cold chain interruption *Iran J Pharm Res* 12 139-144

Kalita D, Mandal M and Mahanta C L 2015. Characterization of litchi juice fermented by probiotic lactic acid bacteria *Int J Food Nutr sci* 4 181-186

Kalliomäki M, Salminen S, Arvilommi H, Kero P, Koskinen P and Isolauri E 2001. Probiotics in primary prevention of atopic disease: A randomised placebo-controlled trial *Lancet* 357 1076-9

Mohammadi R and Mortazavian A M 2011. Review Article: Technological Aspects of Prebiotics in Probiotic Fermented Milks *Food Rev Int* 27 192-21

Mousavi Z E, Mousavi S M, Djomeh Emam Z and Kiani H 2010. Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World J Microbiol Biotechnol* DOI: 10.1007/s11274-010-0436-1

Murcia M A, Opez-Ayerra B L A, Martinez-Tom A M and Vera A M 2000. Industrial processing of broccoli in fruits *Sci Food Agric* 80 1882-1886

Nielsen S S 2010. Food Analysis Laboratory Manual. 2nd ed Springer

Padayatty S J, Katz A, Wang Y, Eck P and Kwon O 2003. Vitamin C as an antioxidant: Evaluation of its role in disease control *J Am Nutr* 22 18-35

Pakbin B, Razavi S H, Mahmoudi R and Gajarbeygi P 2014. Producing probiotic peach juice *Biotech Health Sci* 1 e24683

Panse V G and Sukhatme P V 1957. Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research New Delhi

Pereira A L F, Almeida F D L, Jesus A L T and Rodrigues S 2013. Storage stability of probiotic beverage from cashew apple juice *Food Bioprocess Tech* 6 3155-3165

Pereira A L F, Maciel T C and Rodrigues S 2011. Probiotic beverage from cashew apple juice fermented with *Lactobacillus casei Food Res Int* 44 1276-1283

Pimentel T C, Madrona G S, Garcia S and Prudencio S H 2015. Probiotic viability, physicochemical characteristics and acceptability during refrigerated storage of clarified apple juice supplemented with *Lactobacillus paracasei* ssp. *paracasei* and oligofructose in different package type. *LWT - Food Sci Technol* 63 415-422.

Ranadheera R D C S, Baines S K and Adams M C 2010. Importance of food in probiotic efficacy *Food Res Int* 43 1-7.

Ranganna S 1991. Handbook of Analysis

and Quality Control for Fruits and Vegetable Products. 2nd ed Tata McGraw-Hill Publishing Company Ltd, New Delhi

Rodrigues D, Sousa S, Gomes A M, Pintado M M, Silva J P, Costa P, *et al.*, 2011. Storage stability of *Lactobacillus paracasei* as free cells or encapsulated in alginate-based microcapsules in low pH fruit juices *Food Bioprocess Technol* DOI: 10.1007/s11947-011-0581-z

Sarvari F, Mortazavian A M and Fazeli M R 2014. Biochemical characteristics and viability of probiotic and yogurt bacteria in yogurt during the fermentation and refrigerated storage *Applied Food Biotechnol* 1 53-59.

Saxena V, Mishra G, Saxena A and Vishwakarma K K 2013. A comparative study on quantitative estimation of tannins in *Terminalia chebula, Terminalia belerica, Terminalia arjuna* and *Saraca indica* using spectrophotometer *Asian J Pharm Clin Res* 6 148-149

Shah N P 2001. Functional foods from probiotics and prebiotics *Food Technol* 55 46-53

Shah N P, Ding W K, Fallourd M J and Leyer G 2010. Improving the stability of probiotic bacteria in model fruit juices using vitamins and antioxidants *J Food Sci* 75 278-282

Sheehan V M, Ross P and Fitzgerald G F 2007. Assessing the acid tolerance and the technological robustness of probiotic cultures for fortification in fruit juices *Innov Food Sci Emerg Technol* 8 279-284

Singleton V L and Rossi J A J 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents *Amer J Enol Viticult* 16 144-158

Syed H M, Syed I, Deshpande H W, Kulkarni K D and Kulkarni D N 2007. Chemical Analysis of Food Samples – Laboratory Manual. Needs Agencies, Parbhani (Maharashtra) India.

Tajchakavit S, Boye J I, Belanger D and Couture R 2001. Kinetics of haze formation and factors influencing the development of haze in clarified apple juice *Food Res Int* 34 431-440

Weaver M C, and Daniel R J 2005. The Food Chemistry Laboratory - A Manual for Experimental Foods, Dietetics, and Food Scientists. 2nd ed CRC Press

Yoon K Y, Woodams E E and Hang Y D 2004. Probiotication of tomato juice by lactic acid bacteria *J Microbiol* 42 315-318

Yoon K Y, Woodams E E and Hang Y D 2005. Fermentation of beet juice by beneficial lactic acid bacteria. *Lebensm-Wiss u-Technol* 38 73-75

Yoon K Y, Woodams E E and Hang Y D 2006. Production of probiotic cabbage juice by lactic acid bacteria *Bioresour Technol* 97 1427-1430