Int.J.Curr.Microbiol.App.Sci (2018) *7*(8): 4160-4173

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 08 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.708.435

Fermentation of Pomegranate Juice by Lactic Acid Bacteria

N. Shubhada[1], D.L. Rudresh[2*], S.L. Jagadeesh[1], D.P. Prakash[3] and S. Raghavendra[4]

[1]Department of Post-Harvest Technology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India
[2]Department of Agricultural Microbiology, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India
[3]Department of Fruit Science, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India
[4]Department of Plant biochemistry, College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

**Corresponding author*

Keywords

Fermentation, Kokum juice, Lactic acid bacteria, Pomegranate juice

Article Info

Accepted:
22 July 2018
Available Online:
10 August 2018

A B S T R A C T

This study was undertaken to develop the fermented pomegranate beverage using probiotic lactic acid bacteria and to study the storage stability and biochemical properties of fermented pomegranate beverage. Pomegranate juice alone and blended with different proportion of kokum juice was inoculated with a 24 hr old lactic acid bacteria culture and incubated at 37°C for 72 hr. Bio-chemical changes in pH, TSS, acidity, antioxidant activity, total phenol content and lactic acid bacterial survival at cold storage (4ºC) conditions were analyzed. The results indicated that the fermented pomegranate juice with and without kokum juice fermented by lactic acid bacteria reduced the pH and enhanced the acidity, antioxidant activity, total phenol content. Lactic acid bacterial population reduced during storage period in the fermented beverages. Overall acceptability by Organoleptic / Sensory evaluation of fermented pomegranate beverage with respect to nine point hedonic scale showed that fermented beverage with 15% blend of kokum juice showed highest scores than un-inoculated pomegranate juice (7.55 out of 10).

Introduction

Fermentation is one of the oldest forms of food preservation technology in the world.

The term fermentation was used for the production of wine in early days, but at present it encompasses the foods made by the application of microorganisms including lactic acid bacteria (LAB). There is high potential for the development of blended fermented beverage using different fruit juice.

Keeping the above facts in mind, a lab experiment was conducted at college of horticulture, Bagalkot to investigate the effect of fermentation of pomegranate (*Punica granatum* L.) juice with kokum rind extract (*Garcinia indica* choisy) blend using probiotic lactic acid bacteria.

Materials and Methods

The experiment was laid out in a two factorial completely randomised design. Initially there were thirteen treatments of different combinations of juices (100% pomegranate juice, 85%+15%, 75%+ 25%, 65%+35% pomegranate and kokum juice respectively) fermented with lactic acid bacterial strains (*Lactobacillus acidophilus, L. plantarum*, and *L. delbrueckii*) and three replications. Best seven treatments along with the control were selected based on sensory evaluation which was taken for further storage studies at 4°C for 45 days and analysed for acid content, pH, sugar content, antioxidant activity, phenolic content and microbial load.

The extracted pomegranate and kokum fruit juices were blended wherever needed in the treatments. TSS (Total soluble solids) was adjusted to 18° brix by adding cane sugar using digital refractometer. Juice was pasteurised at 70°C for 5 min. and cooled. All the treatments (except T_1) were inoculated with lactic acid bacterial culture (5% v/v) as per the treatment details. Inoculated treatments were incubated at 37°C for 72 hr. After three days of fermentation the fermented juices was filtered through muslin cloth and the filtrate was filled in sterilized glass bottles. All the treatments were stored in refrigerator (4°C). Juice without inoculation was taken as control.

Factor-I: Treatments

T_1 - Uninoulated Pomegranate juice (Control)

T_3 - 100 % Pomegranate juice + *Lactobacillus plantarum*

T_5 - 85 % Pomegranate juice + 15% Kokum juice + *Lactobacillus acidophilus*

T_6 - 85 % Pomegranate juice + 15% Kokum juice + *Lactobacillus plantarum*

T_7 - 85 % Pomegranate juice + 15% Kokum juice + *Lactobacillus delbrueckii*

T_8- 75 % Pomegranate juice + 25% Kokum juice + *Lactobacillus acidophilus*

T_{10}- 75 % Pomegranate juice + 25% Kokum juice + *Lactobacillus delbrueckii*

T_{11} - 65 % Pomegranate juice + 35% Kokum juice + *Lactobacillus acidophilus*

Factor-II: Storage period (45 days)

S_1 - Initial
S_2 - 15 days
S_3 - 30 days
S_4 - 45 days

Citric acid and lactic acid (%)

A known volume of sample (2ml) was taken and filtered through muslin cloth and volume was made up to 100 ml with distilled water.

From this, five ml of aliquot was taken and titrated against standard NaOH (0.1N) using phenolphthalein indicator.

The appearance of light pink colour indicated the end point. The values were expressed in terms of citric acid and lactic acid as per cent titrable acidity of beverages (Anon., 1984).

$$\text{TA (\%)} = \frac{\text{TV x Normality of NaoH x Equivalent weight of acid x Volume made up x 100}}{\text{Volume of sample taken x Weight of sample x 1000}}$$

Where, TV is Titre value

pH

pH of the samples were measured using digital pH meter. Standard buffer solutions of pH 4.0, 7.0 and 10.0 were used to calibrate the instrument (Jackson, 1973).

Total soluble solids (%)

The total soluble solids (TSS) in samples were measured by using digital refractometer and expressed as ° brix.

Antioxidant activity (%)

The percentage of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity of the samples was determined by a method described by Kathiravan *et al.*, (2014). The hydrogen atom or electron donation abilities of the juice were measured from the bleaching of a purple-coloured methanol solution of stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). A known volume of sample (0.1 ml) or 0.1 ml of methanol (control) mixed with 2.9 ml of 0.004 % DPPH solution (10 mg in 250 ml of methanol prepared freshly) and methanol used as a blank. The mixture was vortexed thoroughly for 1 min and left at 37°C temperature for 30 minutes in darkness and then the spectrophotometer absorbance was read against blank at 517 nm (Model: UV Spectrophotometer, Spectronic ^{R} Genesys ^{TM} 2 Instruments, USA). DPPH free radical scavenging ability (%) was calculated using the formula:

(A 517 nm of control – A 517 nm of sample / A 517 nm of control) × 100

Total phenol (mg GAE/ 100 ml)

Total phenol content of samples was estimated by Folin Ciocalteu reagent (FCR) method (Sadasivam and Manickam, 2005). A sample of 0.5 ml was taken and 10 ml of ethanol was added and filtered the solution using filter paper from which one ml filtered solution was taken in a test tube and boiled at 100°C till the solution was evaporated. One ml of distilled water was added to the test tube and from this 0.5 ml solution was taken into another test tube to which 2.5 ml of distilled water, 1 ml of FCR reagent and 2 ml of sodium carbonate was added and boiled in water bath for 10 minutes. Then the contents of the test tubes were cooled and the absorbance was measured at 650 nm by using spectrophotometer. Total phenol content was calculated with the help of standard graph and expressed in milligram gallic acid equivalents per hundred grams.

Microbial analysis

Microbial count

After fermentation, the samples were subjected for microbiological analysis for lactic acid bacterial counts by employing standard dilution plate count method (Hoben and Somasegaran, 1982).

Dilution

A serial dilution technique was carried out to estimate the lactic acid bacterial (LAB) load in the fermented beverages. One milliliter of the sample was transferred to the test tube containing nine millilitre of distilled water. The test tube was vortexed with the help of spinix cyclomixer. Dilutions up to 10^{-6} were prepared for LAB counts.

The MRS (deMann, Rogosa and sharpe) agar media was used to enumerate LAB count in fermented beverage.

Enumeration

The media was sterilised in the autoclave at 121°C for 20 minutes. In each sterilised petri dish, 1 ml of respective sample was transferred; 25 ml of media was poured in duplicate plates. The plates were rotated both clock and anti-clock wise direction for uniform mixing of the sample and media. After solidification the plates were kept upside down position incubated at 35-37°C for three days.

Counting

The colonies were counted and the total counts were expressed as colony forming unit (cfu) per millilitre of fermented beverages

Sensory evaluation

Sensory evaluation of fermented beverage was carried out by 15 semi trained panel consisting of Teacher and Post graduate students of college of horticulture, Bagalkot with the help of nine point hedonic rating scale (1=dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5=neither like nor dislike, 6= like slightly, 7= like moderately, 8= like very much and 9 = like extremely). The products along with the control were coded and served randomly to the panellist for sensory evaluation immediately after fermentation and up to 45 days at 15 days intervals.

Statistical analysis

The data on the sensory evaluation of experiment I was analysed according to completely randomised design (CRD). The data on the physico-chemical parameters and sensory evaluation of experiment II and III were analysed according to factorial completely randomised design (FCRD). Statistical analysis was performed using Web Agri Stat Package (WASP) Version 2 (Jangam and Thali, 2010). The level of significance used in 'F' and 't' test was p=0.01. Critical difference values were calculated whenever F test was significant.

Results and Discussion

The experiment was conducted to know the biochemical properties and storage stability of different treatments. Based on biochemical, sensory and microbial properties best treatment was selected.

Citric acid and lactic acid

The highest citric acid and lactic acid was recorded in T_{11} (1.64% and 2.35% respectively) and the lowest in T_1 (0.33% and 0.06% respectively). Acid content of fermented beverage increased up to 30 days of storage and afterwards found decreased up to 45 days. However, in uninoculated beverage (control) citric and lactic acid content followed decreasing trend as the storage period advanced. Significantly, the highest citric acid (0.99%) and lactic acid content (1.45%) was observed at 30 DAS. The least citric acid and lactic acid content was observed at initial period (0.89% and 1.24% respectively). The interaction between the treatments and storage period were found to be significantly different. The maximum citric acid content was noted in $T_{11}S_3$ (1.71%) which was on par with T_8S_3 (1.70%) and $T_{11}S_4$ (1.69%).The least was observed in T_1S_4 (0.26%). The highest lactic acid content was recorded in $T_{11}S_3$ (2.42%) which was on par with $T_{11}S_2$ (2.34%) and T_8S_3 (2.29%).

Analysis of acid content in the fermented beverage is necessary to ensure the quality of the beverage. The increase in the citric acid equivalent and a concomitant increase in lactic acid after fermentation (initial period of storage) and during further storage period might be due to the metabolic activity of the probiotic LAB as reported by Tayo and Akpeji (2016). The increase in citric acid and lactic acid content was observed in all the fermented juices up to 30 days of storage. This result was similar to the study conducted by many researchers (Sapna *et al.*, 2002; Nosrati *et al.*, 2014). Moraru *et al.*, (2007) also reported that changes in the pH of the medium and lactic acid development are due to the production of organic acid by LAB culture. However, the acidity of uninoculated juice decreased as the storage period advanced. The decrease in the acidity of the uninoculated juice could be

attributed to chemical interaction between organic constituents of the beverage induced by temperature and action of enzymes as observed by Palaniswamy and Muttuhrishan (1974). Higher citric acid and lactic acid content was observed in 30 DAS (0.99% and 1.45% respectively). After 30 days of storage, marginal decrease in citric and lactic acid content was observed in fermented juices which might be due to the lower metabolic activity of LAB (Table 1 and 2).

pH

The lowest pH was recorded in T_{11} (2.48) followed by T_8 (2.55), T_5 (2.56), T_6 (2.92) and the highest in T_1 (3.54). The result indicated that fermentation by LAB strains resulted in increased acidity of the juice. pH of fermented beverage decreased up to 30 days of storage and afterwards increased up to 45 days. However, pH of uninoculated beverage (control) followed increasing trend as the storage period advanced. The lowest pH was recorded at 30 DAS (2.85) followed by 15 DAS and 45 DAS (2.92 each) and the highest at initial period (2.99).The interaction between the treatments and storage period were found to be significantly different. The minimum pH was observed in $T_{11}S_3$ (2.40) which were on par with $T_{11}S_2$, $T_{11}S_4$, T_8S_3 and T_5S_3 (2.48 each). The juices fermented by *Lactobacillus acidophilus* followed by *Lactobacillus plantarum* showed lower pH than *Lactobacillus delbrueckii*. Similar results were obtained by Yoon *et al.,* (2005) in red beet juice fermented by different LAB stains (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus delbrueckii* and *Lactobacillus casei*). This indicates that LAB strains are able to produce acids even at refrigerated temperature (4°C). Decrease in the pH during storage may be due to the microbial activity and lactic acid production. The results obtained are in conformity with the findings of Pereira *et al.,* (2011) in LAB fermented cashew apple juice and Fonteles *et al.,* (2011) in cantaloupe juice. Kalita *et al.,* (2015) reported that conversion of sugar into organic acids during fermentation resulted in decreased pH in litchi juice fermented by *Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactobacillus rhamnosus* (Table 3).

TSS

The lowest TSS was observed in T_{11} (10.51° brix) followed by T_8 (10.98° brix), T_6 (11.09° brix). The highest TSS was observed in T_1 (18.42° brix) followed by T_3 (11.78° brix), T_{10} (11.77° brix). TSS of all treatments decreased as the storage period advanced except in T_1 (control) where increasing trend was observed. Significantly, the lowest TSS was recorded at 45 DAS (11.95° brix) and highest TSS was observed during initial period (12.44° brix). The interaction between the treatments and storage period showed minimum TSS content in $T_{11}S_4$ (10.30° brix) and maximum TSS content in T_1S_4 (18.62° brix) which was on par with T_1S_3 (18.60° brix). The result of the study confirmed that LAB strains were able to grow in fruit matrices which depend on the substrate used, the oxygen content, other nutrients and the final acidity of the fruit matrix. Similar findings were reported by Yoon *et al.,* (2005) in the fermentation of beet juice by beneficial lactic acid bacteria (Table 4).

Antioxidant activity (%)

The highest antioxidant activity was observed in T_6 (77.07%) which was on par with T_3 (75.96%) and the lowest was noted in T_1 (59.05%). Fermentation by *Lactobacillus plantarum* resulted in higher antioxidant activity with no significant difference between 100 per cent pomegranate and 85 per cent pomegranate juice with 15 per cent kokum juice. The antioxidant activity of fermented beverage with different proportion of fruit

juice and LAB was higher than unfermented pomegranate juice. 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 free hydroxyl groups and relevant aglycones (Mousavi *et al.,* 2013) which might be contributed to the improved antioxidant properties of the fermented juice. El-Nawawy *et al.,* (2009) reported that the antioxidant activity of fermented permeate with natural fruit juices (Guava, mango and lemon juice) was higher when compared to fermented permeate without fruit juices. Similar results were also obtained by Kalita *et al.,* (2015) in litchi juice fermented by probiotic lactic acid bacteria, Mousavi *et al.,* (2013) in pomegranate juice using LAB strains and in *Phyllanthus emblica* fruit juice fermented using probiotic bacterium *Lactobacillus paracasei* (Peerajan *et al.,* 2016). Significantly, the highest antioxidant activity was recorded at initial period (77.60%) and the least at 45 DAS (63.69%). The interaction between the treatments and storage period were found to be significantly different. The maximum antioxidant activity was recorded in T_6S_1 (84.60%) which was on par with T_5S_1 (83.16%), T_3S_1 (82.02%) and T_8S_1 (81.72%).

These results are in conformity to the studies conducted by Filannino *et al.,* (2013) in organic pomegranate juice fermented by *Lactobacillus plantarum* and Khatoon and Gupta (2015) in sweet lime and sugarcane juice fermented using *Lactobacillus acidophilus*. Ascorbic acid is a powerful antioxidant in fruits and can contribute to the antioxidant potential of juices as reported by Reddy *et al.,* (2010). The same authors also reported that improvements in the radical scavenging effect can be related to the increase in the free form of phenolic compounds (Table 5).

Table.1 Changes in citric acid (%) content of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	MEAN
100% UPJ	0.42	0.33	0.30	0.26	**0.33**
100% PJ + *Lp*	0.69	0.79	0.81	0.70	**0.75**
85% PJ + 15% KJ + *La*	0.71	0.76	0.78	0.75	**0.75**
85% PJ + 15% KJ + *Lp*	0.73	0.78	0.80	0.76	**0.77**
85% PJ + 15% KJ + *Ld*	0.55	0.57	0.59	0.55	**0.57**
75% PJ + 25% KJ + *La*	1.51	1.63	1.70	1.68	**1.63**
75% PJ + 25% KJ + *Ld*	1.01	1.16	1.27	1.05	**1.31**
65% PJ + 35% KJ + *La*	1.53	1.65	1.71	1.69	**1.64**
MEAN	**0.89**	**0.96**	**0.99**	**0.93**	
	SEm±		**CD (1%)**		
Treatment	0.007		0.02		
Storage period	0.005		0.02		
Interaction (T× S)	0.01		0.05		

Table.2 Changes in lactic acid (%) content of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	MEAN
100% UPJ	0.07	0.06	0.06	0.05	**0.06**
100% PJ + *Lp*	0.97	1.19	1.24	1.22	**1.15**
85% PJ + 15% KJ + *La*	0.98	1.22	1.31	1.29	**1.20**
85% PJ + 15% KJ + *Lp*	1.03	1.25	1.35	1.33	**1.24**
85% PJ + 15% KJ + *Ld*	0.78	0.91	0.94	0.92	**0.89**
75% PJ + 25% KJ + *La*	2.13	2.23	2.29	2.28	**2.23**
75% PJ + 25% KJ + *Ld*	1.77	1.91	1.98	1.97	**1.90**
65% PJ + 35% KJ + *La*	2.24	2.34	2.42	2.40	**2.35**
MEAN	**1.24**	**1.39**	**1.45**	**1.43**	
	SEm±		**CD (1%)**		
Treatment	0.01		0.07		
Storage period	0.01		0.04		
Interaction (T× S)	0.03		0.14		

Table.3 Changes in pH of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	MEAN
100% UPJ	3.41	3.48	3.60	3.67	**3.54**
100% PJ + *Lp*	3.25	3.16	3.05	3.15	**3.15**
85% PJ + 15% KJ + *La*	2.66	2.58	2.48	2.53	**2.56**
85% PJ + 15% KJ + *Lp*	3.04	2.94	2.84	2.88	**2.92**
85% PJ + 15% KJ + *Ld*	3.21	3.12	3.00	3.09	**3.11**
75% PJ + 25% KJ + *La*	2.61	2.55	2.48	2.55	**2.55**
75% PJ + 25% KJ + *Ld*	3.17	3.04	2.98	3.01	**3.05**
65% PJ + 35% KJ + *La*	2.56	2.48	2.40	2.48	**2.48**
MEAN	**2.99**	**2.92**	**2.85**	**2.92**	
	SEm±		**CD (1%)**		
Treatment	0.01		0.04		
Storage period	0.007		0.02		
Interaction (T× S)	0.02		0.08		

Table.4 Changes in TSS content of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	MEAN
100% UPJ	18.08	18.39	18.6	18.62	**18.42**
100% PJ + *Lp*	12.22	11.97	11.51	11.44	**11.78**
85% PJ + 15% KJ + *La*	11.63	11.22	10.97	10.90	**11.18**
85% PJ + 15% KJ + *Lp*	11.58	11.14	10.88	10.77	**11.09**
85% PJ + 15% KJ + *Ld*	11.84	11.56	11.34	11.31	**11.51**
75% PJ + 25% KJ + *La*	11.42	11.16	10.76	10.58	**10.98**
75% PJ + 25% KJ + *Ld*	11.94	11.78	11.68	11.66	**11.77**
65% PJ + 35% KJ + *La*	10.81	10.55	10.39	10.30	**10.51**
MEAN	**12.44**	**12.22**	**12.01**	**11.95**	
	SEm±		**CD (1%)**		
Treatment **Storage period** **Interaction (T× S)**	0.01 0.007 0.02		0.03 0.02 0.07		

Table.5 Changes in antioxidant activity (%) of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

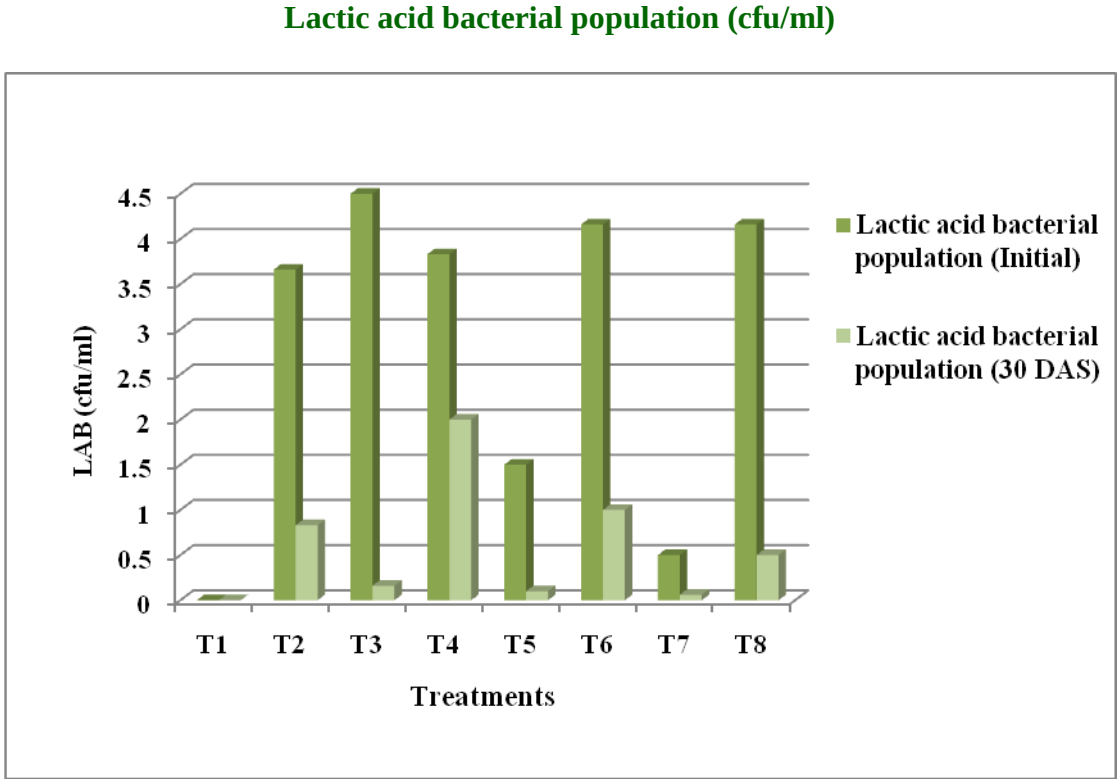
Treatments	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	MEAN
100% UPJ	63.94	61.26	57.53	53.48	**59.05**
100% PJ + *Lp*	82.02	78.22	75.41	68.17	**75.96**
85% PJ + 15% KJ + *La*	83.16	78.87	73.41	63.41	**74.71**
85% PJ + 15% KJ + *Lp*	84.6	79.51	74.92	69.27	**77.07**
85% PJ + 15% KJ + *Ld*	75.79	73.27	69.14	65.55	**70.94**
75% PJ + 25% KJ + *La*	81.72	76.16	72.03	64.45	**73.59**
75% PJ + 25% KJ + *Ld*	74.1	72.17	68.03	63.9	**69.55**
65% PJ + 35% KJ + *La*	75.52	72.61	69.41	61.29	**69.71**
MEAN	**77.6**	**74.01**	**69.98**	**63.69**	
	SEm±		**CD (1%)**		
Treatment **Storage period** **Interaction (T× S)**	0.43 0.30 0.86		1.62 1.14 3.24		

Table.6 Changes in total phenol content (mg GAE/100 ml) of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	MEAN
100% UPJ	233.01	230.43	224.85	220.64	**227.23**
100% PJ + *Lp*	254.25	251.79	247.81	244.87	**249.68**
85% PJ + 15% KJ + *La*	253.16	250.04	248.46	244.03	**248.92**
85% PJ + 15% KJ + *Lp*	256.74	252.58	250.63	248.07	**252.00**
85% PJ + 15% KJ + *Ld*	252.61	249.93	247.22	244.54	**248.57**
75% PJ + 25% KJ + *La*	246.76	242.27	236.26	230.82	**239.03**
75% PJ + 25% KJ + *Ld*	243.23	240.61	238.97	237.45	**240.06**
65% PJ + 35% KJ + *La*	246.81	243.89	241.88	240.73	**243.32**
MEAN	**248.32**	**245.19**	**242.01**	**238.89**	
	SEm±		**CD (1%)**		
Treatment	0.41		1.54		
Storage period	0.29		1.09		
Interaction (T× S)	0.82		3.08		

Table.7 Organoleptic evaluation for overall acceptability of fermented pomegranate beverage with and without kokum juice as influenced by treatments and storage period

Treatments	S_1 (Initial)	S_2 (15DAS)	S_3 (30DAS)	S_4 (45DAS)	MEAN
100% UPJ	7.74	7.87	7.33	7.26	**7.55**
100% PJ + *Lp*	7.79	7.76	7.73	7.47	**7.69**
85% PJ + 15% KJ + *La*	7.93	7.78	7.69	7.44	**7.71**
85% PJ + 15% KJ + *Lp*	8.09	7.9	7.82	7.59	**7.85**
85% PJ + 15% KJ + *Ld*	7.82	7.58	7.49	7.38	**7.57**
75% PJ + 25% KJ + *La*	7.89	7.67	7.71	7.49	**7.69**
75% PJ + 25% KJ + *Ld*	7.76	7.54	7.42	7.2	**7.48**
65% PJ + 35% KJ + *La*	7.82	7.61	7.46	7.35	**7.56**
MEAN	**7.85**	**7.71**	**7.58**	**7.40**	
	SEm±		**CD (1%)**		
Treatment	**0.02**		**0.11**		
Storage period	**0.02**		**0.07**		
Interaction (T× S)	**0.05**		**NS**		



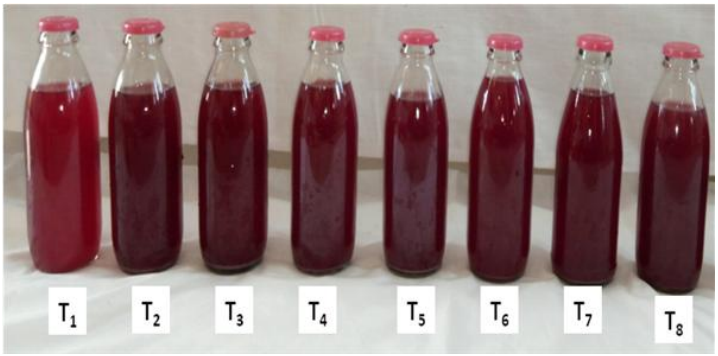


Plate 1: Fermented beverage of pomegranate blended with kokum and control during storage period

Total phenol content (mg GAE/100 ml)

Significantly, the highest total phenol content was recorded in T_6 (252.00 mg GAE/100 ml) followed by T_2 (249.68 mg GAE/100 ml), T_3 (248.92 mg GAE/100 ml), T_5 (248.57 mg GAE/100 ml) and the lowest total phenol content was recorded in T_1 (227.23 mg GAE/100 ml). This result revealed that fermentation process by LAB is good enough to enrich the product with polyphenolic content by selected substrate and starter culture. The release of a significant amount of phenolic content is possible by blending of 85 per cent pomegranate juice with 15 per cent of kokum juice fruits by *Lactobacillus plantarum* mediated fermentation. In case of storage period, maximum score of total phenol was recorded at initial period (248.32 mg GAE/100 ml) and lowest score was

recorded at 45 DAS (238.894 mg GAE/100 ml). In case of interaction effect between treatments and storage period, the highest total phenol content was recorded in T_6S_1 (256.74 mg GAE/100 ml) which was on par with T_3S_1 (254.25 mg GAE/100 ml). The least was observed in T_1S_4 (220.64 mg GAE/100 ml). The decrease in the total phenol content during storage period is probably due to the enzymatic oxidation of polyphenolic content by polyphenol oxidase (Altunkaya and Gokmen, 2008). These findings are in accordance with the results obtained in *Lactobacillus paracasei* HII01 mediated fermentation in *Phyllanthus emblica* fruit juice by Peerajan *et al.,* (2016). Several studies reported that phenolics in the fruit significantly contributed to their antioxidant properties (Shan *et al.,* 2005) (Table 6)

Lactic acid bacterial population (cfu/ml)

The highest lactic acid bacterial population were obtained in T_6 (2.91×10^6 cfu/ml) which was on par with T_8 (2.58×10^6 cfu/ml) followed by T_5 and T_{11} (2.33×10^6 cfu/ml each). Lactic acid bacterial population was not detected in uninoculated pomegranate juice. The survival of *Lactobacillus* spp. varied due to the probiotic strain used as a result of different sensitivity to environmental stresses of bacteria such as low pH and high titratable acidity (Mortazavian *et al.,* 2006).

Lactic acid bacterial population were reduced drastically as the storage period advanced. Significantly, the highest population was observed at initial period (2.79×10^6 cfu/ml) and the least at 30 DAS (0.58×10^6 cfu/ml). During storage, highest LAB population was detected at initial period (2.79×10^6 cfu/ml) and lowest at 30 DAS (0.58×10^6 cfu/ml). Juices fermented by *Lactobacillus acidophilus* showed higher population during initial period of storage but after 30 days population reduced drastically which may be due to higher acidity of the juice produced by *Lactobacillus acidophilus*. Among interactions, the highest population was observed in T_5S_1 (4.50×10^6 cfu/ml) which was on par with T_6S_1 (3.83×10^6 cfu/ml). However, after 30 days of storage, highest population was observed in T_6S_2 (2×10^6 cfu/ml) followed by T_8S_2 (1×10^6 cfu/ml). Among fermented beverages, the lowest population was recorded in $T_{10}S_2$ (0.06×10^6 cfu/ml). The results of this study confirm the findings of Sheehan *et al.,* (2007) indicating that the pH decreased with time and led to a faster decrease in the number of viable bacteria in fruit juices fortified with probiotic lactic acid bacteria. Yanez *et al.,* (2008) reported that an increase in acidity as a result of the fermentation process can reduce the survivability the probiotic lactic acid bacteria. Therefore, variations in bacterial stability observed in this study may be due to pH, fruit juice composition or oxygen present. Similar results were obtained by Ozcan *et al.,* (2015) in fruit based (apple and bluberry) fermented dairy beverages made with *Lactobacillus acidophilus* and *Lactobacillus rhamnosus.* The result of this study was in accordance with the findings of Yoon *et al.,* (2004) in fermented tomato juice. Dogahe *et al.,* (2015) reported that in pineapple, apple and mango juice mixture LAB population was reduced after two weeks during storage at 4°C temperature. Pakbin *et al.,* (2014) reported that *Lactobacillus plantarum* and *Lactobacillus delbrueckii* were capable of surviving in the conditions of low pH and high acidity in fermented peach juice during cold storage (four weeks) at 4°C. In the present study, *Lactobacillus plantarum* showed highest population after 30 days of storage in T_6 (85 % PJ + 15% KJ + *Lactobacillus plantarum*; 2.00×10^6 cfu/ml).

Overall acceptability

Irrespective of storage periods, the mean overall acceptability score of the treatments ranged between 7.48 and 7.85. Highest score

was recorded in T_6 (7.85) and the least score in T_{10} (7.48). The effect of storage period on the overall acceptability of beverage was found to be significant. Maximum score was recorded initial period (7.85) and least score was observed at 45 DAS (7.40). In the interaction between the treatments and storage period, the highest score for overall acceptability was recorded in T_6S_1 (8.09). The lowest score was observed in $T_{10}S_4$ (7.20) (Table 7).

The physico-chemical analysis showed intensification of red colour with the addition of the probiotic LAB, which was not detected in the sensory evaluation. All fermented beverages were acceptable for the colour at the same level. Fruit fibres and flavour compounds might contribute to the desired flavour of the final product. A tendency of higher scores for beverages fermented by *Lactobacillus plantarum* was observed. However, the flavour of the juice fermented by *Lactobacillus delbrueckii* was less appreciated compared to the juice fermented by the *Lactobacillus plantarum* and *Lactobacillus acidophilus*. A fermented dairy taste and flavour were received by panellists as the result of fermentation by LAB. Similar results were obtained by Luckow and Delahunty (2004) in fermented blackcurrant juice in which the authors reported that the sensory characteristic of juice was perfumery, dairy in odour, sour and savoury in flavour. Furthermore production of lactic acid by Lactobacillus may have reinforced the sweet in mouth feeling. In the present study, the overall acceptability was strongly correlated with the taste and flavour but not with the visual appearance. Pimentel *et al.,* (2015) reported that the sensory characteristic of fermented clarified apple juice was dairy in odour and sour in flavour.

From the above experiment it was found that *Lactobacillus plantarum* found to be the best for fermentation of pomegranate juice with 15 per cent kokum juice with respect to enhancement of nutrients, sensory and microbial properties. It was found that beverages at initial period of the storage showed more acceptability by panellists. Quality of the fermented beverage depends upon the substrate and strains used. Besides, the fermentation process by LAB was able to preserve the juice for 45 days under cold storage without any additive addition.

References

Altunkaya, A. and Gokmen, V., 2008, Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa). Food chem.*, 107: 1173-1179.

Dogahe, K. H. M., Darani, K. K., Tofighi, A., Dadgar, M. and Mortazavian, A. M., 2015, *British Biotechnol. J.*, 5(1): 37-50.

El-Nawawy, M. A., El-Malkey, W. and Aumara, I. E., 2009, Production and properties of antioxidative fermented probiotic beverages with natural fruit juices. *Annals Agri. Sci.*, 54(1): 121-135.

Finlannino, P., Azzi, L., Cavoski, I., Vincentini, O., Rizzello, C. G., Gobbetti, M. and Di Cagno, R., 2013, Exploitation of the health-promoting and sensory properties of organic pomegranate (*Punica granatum* L.) juice through lactic acid fermentation. *Int. J. of Food Microbiol.*, 163: 184-192.

Fonteles, T. V., Costa, M. G. M., Ana Jesus, L. T. D. and Rodrigues, S., 2011, Optimization of the fermentation of cantaloupe juice by *Lactobacillus casei* NRRL B-442. *Food Bioprocess Technol.*, 455-760.

Hoben, H. J. and Somasegaran, P., 1982, Composition of the pour, spread and

drop plate methods for enumeration of *Rhizobium* spp. in inoculants made from pre-sterilized peat. *Appl. Environ. microbiol*., 14: 1246-1247.

Jackson, M. L., 1973, Soil chemical analysis. Prentise hall of India Pvt. Ltd., New Delhi.

Jangam, A. K. and Thali, P., 2010, ICAR Research Complex, Goa, Ela, Old Goa, (India).

Kalita, D., Mandal, M. and Mahanta, C. L., 2015, Characterisation of litchi juice fermented by probiotic lactic acid bacteria. *Intr. J. Food Nutr. Sci.,* 4(4).

Kathiravan, T., Nadanasabapathi, S. and Kumar, R., 2014, Pigments and antioxidant activity of optimised Ready-to-Drink (RTD) beetroot (*Beta vulgaris L*.) – passion fruit (*Passiflora edulis var. flavicarpa)* juice blend. *Croat. J. Food Sci. Technol*., 7(1).

Khatoon, N. and Gupta, R. K., 2015, Probiotics beverages of sweet lime and sugarcane juices and its physiochemical, microbiological and shelf- life studies. *J. Pharmacognosy and Phytochemistry,* 4(3): 25-34.

Lucknow, T. and Delahunty, C., 2004. Which juice is 'healthier'? A consumer study of probiotic non-dairy juice drinks. *Food Quality and Preference*, 15: 751-759.

Moraru, D., Blanca, I. and Segal, R., 2007, Probiotic vegetable juices. *Food Technol*., 4: 87–91.

Mortazavian, A. M., Ehsani, M. R., Mousavi, S. M., Sohrabcvandi, S. and Reinheimer, J. A., 2006, Combined effects of temperature-related variables on the viability of probiotic microorganisms in yogurt. *Australian J. of Dairy Sci*., 61: 248-252.

Mousavi, Z. E., Mousavi, S. M., Razavi, S. H., Hadinejad, M., Emam-Djomeh, Z. and Mirzapour, M., 2013, Effect of fermentation of pomegranate juice by *Lactobacillus plantarum* and *Lactobacillus acidophilus* on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. *Food Biotechnol.*, 27: 1–13.

Nosrati, R., Hashemiravan, M. and Talebi, M., 2014, Fermentation of vegetables juice by probiotic bacteria. *Int. J. of Biosci.*, 4(3): 171-180.

Ozcan, T., Yilmaz, E. L., Akpinar, B. A., Delikanli, B. and Barat, A., 2015, Survival of *Lactobacillus* Spp. in fruit based fermented dairy beverages. *Int. J. Food Eng*., 1(1).

Pakbin, B., Razavi, S. H., Mahmoudi, R. and Gajarbeygi, P., 2014, Producing probiotic peach juice. *Biotech Health Sci.*, 1(3): 24683.

Palaniswamy, K. P. and Muthukrishnan, C. R. 1974, Studies on physico-chemical character of lemon juice and squash during storage. *Indian Food Packer*., 28(4): 37-41.

Peerajan, S., Chaiyasut, C., Sirilun, S., Chaiyasut, K., Kesika, P., and Shivamaruthi, B. S., 2016, Enrichment of nutritional value of *Phyllanthus emblica* fruit juice using the probiotic bacterium, *Lactobacillus paracasei* HII01 mediated fermentation. *Fd. Sci. Technol.*, 36(1): 116-123.

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., Prudencio, S. H., 2015, Probiotic clarified apple juice with oligofructose or sucralose as sugar substitutes: Sensory profile and acceptability. *Food sci. and Technol.*, 62: 838-846.

Reddy, L. V., Min, K. H. and Wee, Y. J., 2015, Production of probiotic mango juice by fermentation of lactic acid

Bacteria. *Microbiol. Biotechnol. Lett*., 43(2): 120–125.

Sadasivam, S. and Manickam, A., 2005, *Biochemical Methods*. New Age International (P) Publishers, Second edition. pp. 193-194.

Sapna, V., Vasundhara, M. and Annapurna, M., 2002, Fermented beverages from spices - A new nutraceutical drink. *J. Spices Aromatic Crops*., 11: 106-111.

Shan, B., Cai, Y. Z., Sun, M. and Corke, H., 2005, Antioxidant capacity of 26 spice extracts and characterisation of their phenolic constituents. *J. Agri. Food Chem.*, 53: 7749-7759.

Tayo, B. A. and Akpeji, S., 2016, Probiotic viability, physicochemical and sensory properties of probiotic pineapple juice. *Fermentation*, 2(20).

Yanez, R., Marques, S., Girio, F. M., and Roseiro, J. C., 2008. The effect of acid stress on lactate production and growth kinetics in *Lactobacillus rhamnosus* cultures. *Process Biochem.,* 43; 356–361.

Yoon, K. Y., Edward, E. and Hang, Y. W., 2004, Probiotication of tomato juice by lactic acid bacteria. *J. Microbiol.,* 42: 315—318

Yoon, K. Y., Woodams, C. and Hang, Y. D., 2005, Fermentation of beet juice by beneficial lactic acid bacteria. *Lebensm-wiss. U. Technol*., 38: 73-75.

How to cite this article:

Shubhada, N., D.L. Rudresh, S.L. Jagadeesh, D.P. Prakash and Raghavendra, S. 2018. Fermentation of Pomegranate Juice by Lactic Acid Bacteria. *Int.J.Curr.Microbiol.App.Sci.* 7(08): 4160-4173. doi: https://doi.org/10.20546/ijcmas.2018.708.435