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Organic Additives To Reduce Browning, Microbial Load and Quality Loss of Litchi Juice During Storage

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Abstract

The litchi (*Litchi chinensis*Sonn.) fruit is highly perishable and is subjected to spoilage and browning during storage. Organic acids are water soluble and are capable to control microbial growth through acidic medium developed due to release of H^+.Observations on all biochemical parameters of litchi juice were taken at 0 days, 15 days,30 days, 45 days and 60 days of storage.The salts of various organic acids like citric acid and tartaric acid have been tested forpreservation of litchi juice.The most suitable preservation methods for litchi juice reported duringinvestigation were: Pasteurized juice + 0.05% Potassium meta- bi- sulphite (KMS); Non-pasteurizedjuice under cold storage; Pasteurized juice + 0.25% Citric acid; and Pasteurized juice + 0.25%Tartaric acid.Overall citric acid appears to be the safest food preservative for litchijuice preservation which can be effectively used to inhibit chemical, enzymatic or microbiological deterioration at concentration as low as 0.1- 0.25 %.

Keywords: Browning, Citric acid, KMS, Litchi juice, Microbial count, Tartaric acid.

Introduction

The litchi (*Litchi chinensis*Sonn.) is one of the cheapest fruits of region and belongs tofamily Sapindaceae. The litchi fruit isrich source of vitamins 'C' and phenolic compounds that have antioxidantactivities, but it may decrease after harvest (Seymour*et al.*, 2012;Wall, 2006; Hu *et al.*, 2008).Litchi has very shortharvesting season and fruits are delicate and highly perishable so long duration storage results in pericarpbrowning. Litchi is mostly consumed as a table fruit;however, it can be utilized for processing into different products such as canned

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litchi,squash, cordial, syrup, ready to serve (RTS), soft drink, wine, jam, jelly and concentrates.Processing of litchi fruits also reduces on-season glut in market and results indistribution of fruit products throughout the year. Litchi fruit consists of 60 percent juice, 8 percent rag, 19 percent skin and 16 percent seeds varied depending upon the varieties and the climate under which it is grown.

Litchi juice have large amount of fermentable sugar and acid which are in quiteequilibrium. The fresh litchi juice contains 161.4g total sugar and 2.2 g total acids (Zeng *et al.*, 2008). Thus, juice extracted from litchishould be consumed fresh otherwise fermentation takes place and sour odour gets initiatedresulting in spoilage.The content of vitamin-C was reported as 354.12 mg/l which is 8 times that ofapple juice (40 mg/l) (Yang, 2002). The factors responsible for spoilageof litchi juice include physical, chemical, enzymatic and microbial changes. The presence ofoxygen accelerates deterioration of fruit juices which leads to ascorbic acid degradation,increase in browning and growth of aerobic bacteria and molds (Kennedy *et al.*, 1992; Solomon *et al.*, 1995; Soares and Hotchkiss, 1999).Among different preservatives, sodium and potassium metabisulphites can reducebrowning while, citric and tartaric acids are well known to bring down activityof polyphenol oxidase (Costa *et al.*, 2003;Queiroz *et al.*, 2008).Organic acids like tartaric acid and citric acid, whichare edible, can be added to litchi juice at the rate of 3.0 g/l to make the total organic acid upto5.0g/l and lower the pH below 3.8 which prevent the growth of micro-organisms other thanyeast so good to enhance shelf life during storage.In the present study, an attempt was made to identify suitable organic preservatives for long term storage of litchi juice.

Materials and Methods

The present investigation was conducted in the Postgraduate Horticulture Laboratory, Department of Horticulture, School of Agriculture, Lovely Professional University, Phagwara.The Litchi fruits (Culcuttia variety) of uniform size, disease free were picked at full maturity randomlyfrom all the four directions of the plants. The fruits were sorted and washed, the skins of fruits were removed, and seeds were separated to isolate pulp. Juice wasextracted from pulp by automatic juicer. The whole of the juice was then filtered by using muslin cloth to obtain a clear juice. The pasteurization and preservative treatmentswere applied as per

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treatments: T_1(Pasteurized juice or Control); T_2 (Pasteurized juice + 0.05% Potassium meta- bi-sulphite);T_3(Pasteurized juice +0.1% Citric acid); T_4(Pasteurized juice + 0.15% Citric acid); T_5(Pasteurized juice + 0.20% Citric acid); T_6(Pasteurized juice + 0.25% Citric acid); T_7(Pasteurized juice + 0.1% Tartaric acid); T_8(Pasteurized juice + 0.15% Tartaric acid); T_9(Pasteurized juice + 0.20% Tartaric acid); T_{10}(Pasteurized juice + 0.25% Tartaric acid); and T_{11}(Non-pasteurized juice under cold storage). Observations on Total Soluble Solids (TSS in ^oB), ascorbic acid content (mg/1000 ml) and pH were determined byAssociation of Official Analytical Chemists (AOAC 2005) while sugar content (%) was estimated through method illustrated by Ranganna (2002), consumer acceptability was determined as per the 9-point hedonic scale (Lim, 2011),non-enzymatic browning was observed through spectrophotometer and microbial load was determined as colonies farming units (CFUs). All observations were taken at the interval of 15 days up to two months (D_1: At 15 days of storage, D_2: At 30 days of storage, D_3: At 45 days of storage, D_4: At 60 days of storage). The observations on various parameters were statistically analyzed through factorial CRD (Completely Randomized Design) to test the significance of both the factors, preservative treatments (T) and number of days of storage (D) of litchi juice.

Results and Discussion

Quality of litchi juice at Zero day: The quality parameters of litchi juice supplemented with preservatives was estimated and presented in Table-1. It is evident from the observationsthat TSS was significantly affected by preservatives added in juice. High estimates of TSS was reported in pasteurized fruit juice treated with citric acid or tartaric acid with highest value 16.10^0B and 15.87^0B in T_6 and T_{10}, respectively. This might be associated with thermal hydrolysis of these acids into sugars as confirmedby Deka and Sethi(2001)in mixed fruit juice-spiced beverages.There was no significant variation in Ascorbic acid content of fruit juice under different treatments at 0 days of storage. The acidity was significantly varied between treatments with highest value as 0.336% in T_{11} at 0 day of storage.The lower value of acidity in pasteurized litchi juicemight be due to loss natural acidity during pasteurization.The higher acidity in tartaric acid and citric acid preserved litchi juice might be due to high concentration of H^+ ions released due to dissociation of the acids added as preservatives. Similarly, the effect of preservatives and

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pasteurization on pH was reported to be significant with the highest (4.33) pH in $T_2$and lowest with use of high concentration (0.20 or 0.25%) of citric or tartaric acid as preservatives. Pasteurization is associated with the leaching of organic acids added as preservative and so is responsible for lower pH value and high acidity (Gupta *et al.*, 2011;Gonzalez-Cebrino *et al.*, 2012).The highest total sugar (17.47%), reducing sugar (7.78%) and non-reducing sugar (9.21%) was reported in T_{11} however, lowest (15.17%, 8.12% and 6.62%, respectively) total sugar was reported in T_2. Increase in sugar content with increased level of citric acid and tartaric acid during storage might be associated with better substrate hydrolysis of complex compounds like polysaccharides and even the organic acids added as preservatives. Highest consumer acceptability at all evaluation was reported with non-pasteurized litchi juice under cold storage(T_{11}). However, significantly low acceptability was reported at higher concentration of citric acid and tartaric acid might be due to off flavour developed during pasteurization of litchi juice.

Biochemical parameters of litchi juice during storage: TSS content of fruit juice was significantly influenced by both the factors, number of days of storage (A) and preservatives (B), however interaction effect was not significant. A gradual increase in TSS was reported in litchi fruit juice during storage under all preservative's treatments (Table-2) which might be associated with increased hydrolysis of polysaccharides with gradual passage of time (Bhardwaj, 2013). The increase in TSS was higher in juices stored at ambient temperature in comparison to low temperature storage (T_{11}) which could be attributed to high rate of dissolution of polysaccharides and acids at high temperature (Prasad and Mali, 2000;Bhardwaj and Nandal, 2014). High rate of TSS increase in fruit juice treated with citric acid or tartaric acid was associated with hydrolysis of these acids into sugars (Deka and Sethi, 2001).

Ascorbic acid content of fruit juice was significantly influenced by both the factors and their interaction (Table-2). Ascorbic acid content was reported to decrease down vigorously during storage and highest (from 122.55 mg/100g to 14.77 mg/100g) decrease was reported in T_1(pasteurized juice without preservatives and stored at ambient temperature) whereas lowest (from 132.67 mg/100g to 34.49 mg/110g) decrease in ascorbic acid was observed in T_{11}(non-pasteurized juice without preservatives and stored at low temperature). The expected decrease in

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ascorbic acid content of litchi fruit juice during passage of storage duration might be due to the interaction effect of light, metallic ions and prevailing high temperature which had resulted in oxidation of ascorbic acid to dehydroascorbic acid (Ahmed*et al.,* 2008;Shubhra*et al.,* 2014). Almost all treatments had been reported with about 40±3 percent loss in ascorbic acid content within first 15 days of storage which might be associated with its aerobic oxidation in early phase of storage due to presence of residual oxygen in bottles during packing while later phase of degradation was associated with anaerobic oxidation and thermal break down at ambient temperature (Kabasakalis*et al.,* 2000;Blasco*et al.,* 2004) or in pasteurized juice (Cvetkovic and Jokanovic, 2009). However, non-pasteurized litchi juice preserved under refrigerator condition suffers with lower rate of degradation as low temperature reduces kinetics of ascorbic acid break down (Guo *et al.,* 2015). Further addition of 0.05% percent KMS in pasteurized litchi juice had resulted reduced level of ascorbic acid degradation due to inactivation of enzymes responsible for breakdown of ascorbic acid (Golden*et al.,* 2005; Guo *et al.,* 2015;Leong and Oey, 2012).

Titratable acidity of litchi juice was also significantly affected with storage duration and showed increasing trend during storage in all treatments(Table-2). Among different preservatives higher concentration of both, citric acid and tartaric acid (0.20% and 0.25%) were effectively regulated the increase in acidity but addition of 0.05% KMS was reported to be best for pasteurized bottled juice of litchi at ambient temperature storage. pH content of fruit juice has been reported to correspond the titratable acidity for all treatments and storage period. Further, it was also significantly influenced by interaction effect. At all days of storage, higher acidity in tartaric acid and citric acid preserved litchi juice might be due to high concentration of H+ ions released due to dissociation of the acids added as preservatives whereas other treatments showed low acidity. The increase in acidity during storage could be possibly associated with fermentation of sugar present in litchi juice or hydrolysis of esters (Amerine and Cruess, 1960).

Reducing sugar, total sugarand non-reducing sugarwere reported to get significantly increased during storage (Table-3) in alltreatments however, highest increase in total sugar was recorded in T_1 which showed 45 .39%rise form 15.43% at 0 day to 22.43% at 60 days of storage whereas least (20%) increase inlevel of total sugar was recorded in T_2 (from 15.31% at 0 days to 18.37% at 60 days) followed by 21.05% rise in T_{11}. Increase in sugar content with increased level of

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citric acid and tartaric acid during storage might be associated with better substrate hydrolysis of complex compounds like polysaccharides and even the organic acids added as preservatives due to high temperature in comparison to refrigerator storage of litchi fruit juice in T_{11}(Sarmah*et al.*, 1981;Ranote and Bains, 1982;Pareek *et al.*, 2015). Increase in reducing and total sugar of mandarin juice with advancement of storage period in all treatments including 0.5% KMS might be due to release of SO_2 to create acidic medium for rapid hydrolysis of polysaccharides including pectin, cellulose and starch (Garg*et al.,* 2008; Pareek *et al.,* 2015).

It is confirmed from the observations that acceptability of litchi juice was significantly influenced by various preservative treatments and has been gradually changed during the storage(Table-3). Highest consumer acceptability at all evaluation was reported with non-pasteurized litchi juice under cold storage (T_{11}). However, significantly low acceptability was reported at higher concentration of citric acid and tartaric acid. The lower acceptability of preserved litchi juice in all the treatments except T_{11} might be due to off flavour developed during pasteurization of litchi juice. The off flavour has become more intensified during end of storage period hence significantly reduced the acceptability of litchi juice (Bhardwaj and Mukherjee, 2012;Vijayanand*et al.,* 2010).

Absorbance at 440nm light wavelength: The browning of juice during storage is associated with Maillard reaction between alpha-amino groups and reducing sugar and is measured in terms of absorbance.In the beginning (0, 15 and 30 days of storage), although there was very low degree ofvariation in absorbance, but all treatments had been reported low value of absorbance incomparison to T_{11} which might be associated with leaching of solublecomponents during pasteurization in fruit juice treated with preservatives. The results reflected that only high concentration of tartaric acid and citric acids were able to inhibit the enzymatic browning of preserved litchi juice (Figure 1). The efficient regulation of non-enzymaticbrowning of litchi juice by KMS (T_2) and high concentration of citric (T_6) and tartaric acid(T_{10}) might be due to their ability to prevent oxidation of sugar in the juice as they are supposed toeffectively bind with substrate or enzymes to prevent oxidation (Calder *et al.,* 2011).KMS @ 0.5% (T_2) and low temperature storage (T_{11}) effectively lowered oxidation and so browning of litchi juice (Liu *et al.,* 2012).

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Microbial load of litchi fruit juice: The bacterial colonies were not detectable till 15 days of storage of litchi juice under all treatments. At 30 days of storage bacterial colonies were recorded only in juice without any preservatives stored at ambient temperature (T_1) while other treatments did not give any sign of bacterial colonies (Table-4). At 30 and 60 days of storage highest number of colonies (0.52± 0.04 and 0.75±0.05, respectively) were recorded in T_1 followed by T_3 (0.51±0.05 and 0.61±0.04, respectively) whereas T_{11} (Non-pasteurized juice stored at 4^0C temperature) had been reported with lowest (0.37±0.02 and 0.53± 0.05, respectively) value in comparison to other treatments. Thus, at 60 days of storage only fruit juice under T_2, T_5, T_6, $T_{10}$and T_{11} was reported to be in safe range (103 to 105 CFU/ml) of microbial count (Rahman *et al.*, 2011). Increase in microbial load during storage of fruit juice under ambient temperature was also reported by Nwachukwu and Ezcigbo (2013). However, reduced microbial growth was reported in fruit juice treated with KMS (Akhtar *et al.,* 2009;Amin *et al.,* 2008;Hashmi*et al.,* 2007;Ogiehor and Ikenebomeh, 2004)and at lower temperature (Chauhan *et al.*, 2002).

Conclusion

Litchi juice which has unique combination of acidity and sugar is highly sensitive to storage temperature, preservative treatments and pre-storage pasteurization treatment. The most suitable preservation methods for litchi juice reported during investigation were:Pasteurized juice + 0.05% Potassium meta- bi- sulphite; Non-pasteurized juice under cold storage; Pasteurized juice + 0.25% Citric acid; and Pasteurized juice + 0.25% Tartaric acid.

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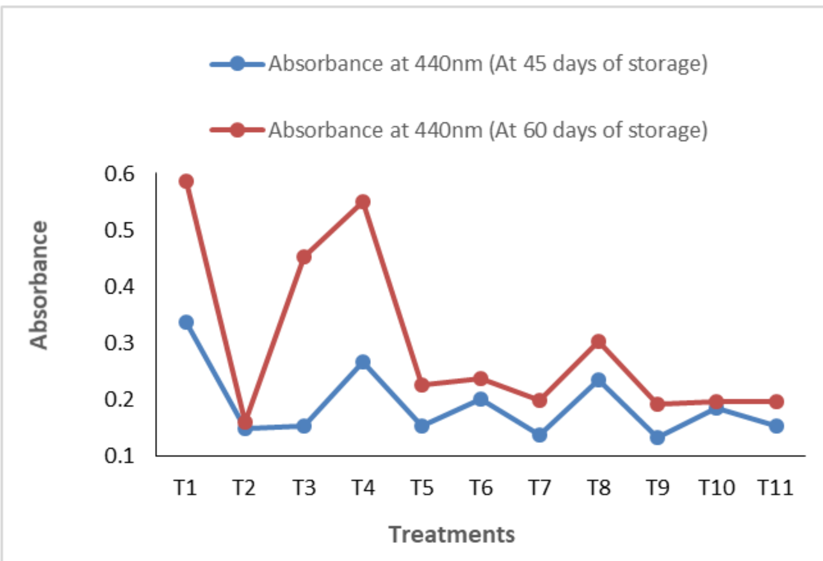

Figure 1. Trend of Absorbance by fruit juice at 440nm wavelength

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Table 1. Quality attributes of litchi fruit juice under different treatments at zero days of storage

Treatments	TSS (^{0}B)	Ascorbic Acid (mg/1000ml)	Titratable Acidity (%)	pH	Total Sugar (%)	Reducing Sugar (%)	Non-reducing Sugar (%)	Overall Acceptability	TSS: Acid	Sugar: Acid
T_1 (Control)	15.00	220.89	0.297	4.27	15.43	6.75	8.24	8.53	50.51	51.95
T_2 (0.05% KMS)**	15.20	219.95	0.287	4.33	15.31	6.69	8.18	8.60	52.96	53.34
T_3 (0.10% Citric acid)	15.23	220.49	0.300	3.23	15.77	6.93	8.40	8.50	50.77	52.57
T_4 (0.15% Citric acid)	15.27	220.15	0.300	3.04	15.58	6.83	8.31	8.13	50.90	51.93
T_5 (0.20% Citric acid)	15.33	221.09	0.308	2.82	16.03	7.06	8.53	7.97	49.77	52.04
T_6 (0.25% Citric acid)	16.10	220.42	0.307	2.64	15.98	7.03	8.50	7.87	52.44	52.05
T_7 (0.10% Tartaric acid)	15.20	220.67	0.292	3.08	15.17	6.62	8.12	8.53	52.05	51.95
T_8 (0.15% Tartaric acid)	15.20	220.39	0.299	2.74	15.56	6.82	8.31	8.30	50.84	52.04
T_9 (0.20% Tartaric acid)	15.33	221.04	0.305	2.54	15.86	6.97	8.44	7.97	50.26	52.00
T_{10} (0.25% Tartaric acid)	15.87	220.59	0.327	2.26	16.99	7.53	8.98	7.87	48.53	51.96
T_{11} (Cold storage) *	15.00	221.01	0.336	3.05	17.47	7.78	9.21	9.00	44.64	51.99
Mean	**15.34**	**220.61**	**0.305**	**3.09**	**15.92**	**7.00**	**8.47**	**8.30**	**50.33**	**52.17**
CD	**0.221**	**NS**	**0.023**	**0.044**	**1.142**	**0.571**	**0.542**	**0.195**	**4.324**	**NS**
SeM (±)	**0.075**	**0.616**	**0.008**	**0.015**	**0.389**	**0.195**	**0.185**	**0.067**	**1.563**	**1.684**

***Litchi juice taken in T_{11} was non-pasteurized while in other treatments pasteurized juice was taken

**KMS: Potassium meta- bi- sulphite

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Table-2: TSS, Ascorbic Acid and Acidity of litchi fruit juice during storage after treatment with different preservatives

Treatments/Days of Storage	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	Mean (D)	Factors	C.D.	SE(m)
	TSS (Total Soluble Solids in 0Brix)														
D_1	15.03	15.20	15.30	15.30	15.37	16.30	15.37	15.27	15.37	15.83	15.10	**15.40**	**Factor (D)**	**0.08**	**0.03**
D_2	15.20	15.33	15.33	15.37	15.40	16.53	15.53	15.27	15.40	15.87	15.13	**15.49**	**Factor (T)**	**0.13**	**0.04**
D_3	15.30	15.60	15.47	15.60	15.60	16.60	15.67	15.53	15.67	16.00	15.20	**15.66**	**Factor (D X T)**	**NS**	**0.09**
D_4	15.33	15.83	15.53	15.80	15.73	16.73	15.73	15.67	16.00	16.13	15.27	**15.80**			
Mean (T)	**15.22**	**15.49**	**15.41**	**15.52**	**15.53**	**16.54**	**15.58**	**15.43**	**15.61**	**15.96**	**15.18**				
	Ascorbic Acid (mg/1000ml)														
D_1	122.55	129.61	125.15	124.81	125.75	127.66	125.19	124.91	125.56	127.92	132.67	**126.52**	**Factor (D)**	**0.38**	**0.14**
D_2	85.80	96.77	86.91	87.41	87.87	92.06	87.06	87.10	88.24	90.54	101.46	**90.11**	**Factor (T)**	**0.63**	**0.22**
D_3	45.89	65.96	50.77	51.83	52.86	61.91	51.19	51.93	52.15	61.72	65.61	**55.62**	**Factor (D X T)**	**1.26**	**0.45**
D_4	14.77	34.84	19.65	20.71	21.74	30.79	20.07	20.81	21.03	30.60	34.49	**24.50**			
Mean (T)	**67.25**	**81.79**	**70.62**	**71.19**	**72.05**	**78.10**	**70.88**	**71.19**	**71.74**	**77.69**	**83.56**				
	Titratable Acidity (%)														
D_1	0.35	0.32	0.35	0.35	0.34	0.34	0.34	0.35	0.34	0.37	0.35	**0.35**	**Factor (D)**	**0.01**	**0.00**
D_2	0.40	0.35	0.39	0.38	0.38	0.37	0.37	0.38	0.38	0.39	0.37	**0.38**	**Factor (T)**	**0.01**	**0.00**
D_3	0.41	0.36	0.40	0.39	0.39	0.38	0.39	0.39	0.39	0.40	0.40	**0.39**	**Factor (D X T)**	**NS**	**0.01**
D_4	0.43	0.38	0.42	0.42	0.41	0.40	0.41	0.41	0.41	0.42	0.42	**0.41**			
Mean (T)	**0.40**	**0.35**	**0.39**	**0.39**	**0.38**	**0.37**	**0.38**	**0.38**	**0.38**	**0.39**	**0.38**				
	pH (Active Acidity)														
D_1	3.74	4.33	3.54	3.32	3.18	2.99	3.58	2.99	2.90	2.59	3.17	**3.30**	**Factor (D)**	**0.04**	**0.02**
D_2	3.06	4.36	3.89	3.74	3.47	3.20	3.82	3.14	3.13	3.03	3.25	**3.46**	**Factor (T)**	**0.07**	**0.03**
D_3	3.49	4.45	4.08	4.03	3.71	3.65	4.03	3.68	3.51	3.34	3.17	**3.74**	**Factor (D X T)**	**0.14**	**0.05**
D_4	3.84	5.27	4.25	4.17	3.95	3.86	4.22	4.20	4.06	3.93	3.10	**4.08**			
Mean (T)	**3.53**	**4.60**	**3.94**	**3.82**	**3.58**	**3.43**	**3.91**	**3.50**	**3.40**	**3.22**	**3.17**				

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Table-3: Sugar content and Acceptability of litchi fruit juice during storage after treatment with different preservatives

Treatments/ Days of Storage	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	Mean (D)	Factors	C.D.	SE(m)
								Total Sugar (%)							
D_1	18.03	16.15	18.37	18.18	17.85	17.46	17.77	18.17	17.87	19.05	17.25	**17.83**	**Factor (D)**	**0.28**	**0.10**
D_2	20.66	16.74	20.04	19.83	19.52	19.08	19.43	19.76	19.54	20.18	17.94	**19.34**	**Factor (T)**	**0.46**	**0.16**
D_3	21.34	17.36	20.71	20.51	20.19	19.76	20.11	20.44	20.21	20.85	19.01	**20.04**	**Factor (D X T)**	**NS**	**0.32**
D_4	22.43	18.37	21.81	21.60	21.29	20.85	21.20	21.53	21.30	21.94	21.15	**21.22**			
Mean (T)	**20.61**	**17.15**	**20.23**	**20.03**	**19.71**	**19.29**	**19.63**	**19.97**	**19.73**	**20.51**	**18.84**				
								Reducing Sugar (%)							
D_1	7.66	7.12	7.76	7.66	7.50	7.30	7.45	7.65	7.51	8.10	7.67	**7.58**	**Factor (D)**	**0.12**	**0.04**
D_2	8.27	7.41	8.59	8.49	8.33	8.11	8.29	8.45	8.34	8.49	8.01	**8.25**	**Factor (T)**	**0.19**	**0.07**
D_3	9.32	7.72	8.93	8.73	8.56	8.45	8.62	8.69	8.51	8.66	8.54	**8.61**	**Factor (D X T)**	**0.39**	**0.14**
D_4	9.87	8.22	9.47	9.28	9.12	9.00	9.17	9.23	9.05	9.04	9.62	**9.19**			
Mean (T)	**8.78**	**7.62**	**8.69**	**8.54**	**8.38**	**8.21**	**8.38**	**8.51**	**8.35**	**8.57**	**8.46**				
								Non-reducing Sugar (%)							
D_1	9.85	8.58	10.09	10.00	9.84	9.65	9.80	9.99	9.85	10.41	9.11	**9.74**	**Factor (D)**	**0.17**	**0.06**
D_2	11.77	8.86	10.88	10.78	10.63	10.42	10.59	10.75	10.64	11.10	9.43	**10.53**	**Factor (T)**	**0.28**	**0.10**
D_3	11.42	9.16	11.20	11.18	11.06	10.75	10.91	11.16	11.12	11.58	9.94	**10.86**	**Factor (D X T)**	**NS**	**0.20**
D_4	11.94	9.64	11.72	11.70	11.56	11.26	11.43	11.68	11.64	12.26	10.96	**11.43**			
Mean (T)	**11.24**	**9.06**	**10.97**	**10.92**	**10.77**	**10.52**	**10.68**	**10.89**	**10.81**	**11.34**	**9.86**				
								Over all Acceptability							
D_1	8.40	8.53	8.10	7.73	7.57	7.47	8.13	7.90	7.57	7.47	9.00	**7.99**	**Factor (D)**	**0.09**	**0.03**
D_2	7.93	8.07	7.70	7.30	7.17	7.07	7.40	7.50	7.10	7.00	8.50	**7.52**	**Factor (T)**	**0.14**	**0.05**
D_3	6.40	7.40	6.87	6.53	6.30	6.20	6.93	6.63	6.33	6.20	7.80	**6.69**	**Factor (D X T)**	**0.28**	**0.10**
D_4	5.40	6.63	6.23	5.93	5.80	5.70	6.00	6.07	5.70	5.63	6.40	**5.96**			
Mean (T)	**7.03**	**7.66**	**7.23**	**6.88**	**6.71**	**6.61**	**7.12**	**7.03**	**6.68**	**6.58**	**7.93**				

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Table 4. Total bacterial counts in litchi juice under different treatments during storage

Treatments	At 30 Days of storage	At 45 Days of storage	At 60 Days of storage
T_1	0.34±0.03	0.52±0.040	0.75±0.050
T_2	ND	0.43±0.050	0.51±0.050
T_3	ND	0.51±0.050	0.61±0.040
T_4	ND	0.48±0.045	0.59±0.050
T_5	ND	0.45±0.050	0.54±0.055
T_6	ND	0.45±0.030	0.55±0.040
T_7	ND	0.49±0.030	0.59±0.020
T_8	ND	0.47±0.030	0.56±0.040
T_9	ND	0.45±0.020	0.57±0.050
T_{10}	ND	0.44±0.040	0.55±0.040
T_{11}	ND	0.37±0.020	0.53±0.050

ND: Nondetectable colonies