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CHARACTERISATION OF LITCHI JUICE FERMENTED BY PROBIOTIC LACTIC ACID BACTERIA

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ABSTRACT

In this research, production of probiotic litchi juice through its fermentation by three strains of lactic acid bacteria: *Lactobacillus plantarum*, *L. Rhamnosus* and *L. acidophilus* were examined. Fermentation was carried out at 37 °C for 72 h under microaerophilic conditions. Changes in pH, titrable acidity, sugar content, microbial population, color, total phenolic content and antioxidant were measured during the fermentation period and the viability of all strains was also monitored during storage at 4 °C for 4 weeks. The results indicate that *L. plantarum* and *L.rhamnosus* lowered the pH at the initial stages of fermentation along with greater sugar consumption than *L. acidophilus* and showed better microbial growth during fermentation. *L. plantarum* and *L. Rhamnosus* showed higher viability during the storage time. Viable cells remained at maximum level up to 2 weeks but decreased dramatically after 4 weeks. Total phenolic content and DPPH radical scavenging studies showed that fermentation of litchi juice using these strains of *Lactobacillus* increased the antioxidant activity with *L. acidophilus* exhibiting highest antioxidant capacity. The results indicated that litchi juice provides scope for use as a suitable medium for delivery of the probiotics on fermentation to enhance the health benefits of the juice.

Keywords: Litchi juice, Probiotic, Fermentation, Lactobacillus

INTRODUCTION

To date many systems have been developed in order to deliver probiotics, including fermented and non-fermented dairy products, fruit juices, emulsions, breakfast cereals, cereal bars, ice creams, cheeses and their derivatives (Antunes, Cazetto, &Bolini, 2005; Cruz, Antunes, Sousa, Faria, &Saad, 2009; dos Santos Leandro, de Araújo, da Conceição, de Moraes, & de Carvalho, 2013; Mantzouridou, Spanou, &Kiosseoglou, 2012; Saarela, Virkajarvi, Alakomi, Sigvart-Mattila, &Matto, 2006; Ying *et al.*, 2013). However, an increased demand for non-dairy probiotic products comes from vegetarianism; milk cholesterol content, milk allergy and other factors (Ray *et al.*, 2009). This fact has led to development of probiotic products from various food matrices including fruits (Prado *et al.*, 2009) and vegetables (Yoon *et al.*, 2009).

Technological advances have made it possible to alter some structural characteristics of fruit and vegetables matrices by modifying food components in a controlled way (Betoret *et al.*, 2009). This could make them ideal substrates for the culture of probiotics, since they already contain beneficial nutrients such as minerals, vitamins, dietary fibers, and antioxidants (Luckow&Delahunty, 2004) while lacking the dairy allergens that might prevent consumption by certain segments of the population (Tuorila&Cardello, 2002). There is a genuine interest in the development of fruit juice based functional beverages with probiotics because they have taste profiles that are appealing to all age groups and because they are perceived as healthy and refreshing foods (Yoon *et al.*, 2004, Tuorila & Cardello, 2002, Sheehan *et al.*, 2007).

Litchi (*Litchi chinensis*Sonn.) is an important tropical fruit largely consumed in the form of processed products such as juice. Litchi is an excellent source of vitamin C (40- 90 mg/100 g) (Menzel, 2002). It also contains 21.6 g/100 g of total sugar (Haq & Rab, 2012), 0.37 g/100 g titratable acidity (as citric acid) (Sun, Liang, Xie, Lei, & Mo, 2010), 43 aroma volatile compounds (Wu, Pan, Qu, &Duan, 2009) and 0.8 mg/g total phenolic compounds (Dajanta, Apichartsrangkoon, &Somsang, 2012). The objectives of this study were to examine the viability and functional properties of probiotic combinations in litchi juice during storage.

MATERIALS AND METHODS

PROBIOTIC STRAIN AND CULTURE PREPARATION

Lactobacillus isolates, *Lactobacillus plantarum* MTCC5422, *L. rhamnosus* MTCC1480, and *L. acidophilus*

MTCC447 were obtained from Microbial Type Culture Collection and Gene (MTCC) (Chandigarh, India) and coded as Lp, Lr and La, respectively. Stock solution was prepared by adding sterile glycerol (50% v/v) to the activated culture. The glycerol stock culture was stored at -20 ^{o}C in sterile screw cap tubes. The identity of all the probiotic bacteria was confirmed using biochemical methods described by Shah and Lankaputhra (1997). The probiotic organisms were grown individually by inoculating into 10 mL sterile de Man Rogosa and Sharp (MRS) broth (Himedia Laboratories Pvt. Ltd, Mumbai, India) and incubated at 37 ^{o}C for 2 days under aerobic condition. The cells were harvested by centrifuging (Sigma, Germany) at 1500 x g for 15 min at 25 ^{o}C. Before inoculation into fruit juices, the harvested cells were washed twice with sterile saline water (0.85% w/v NaCl) to remove any residual MRS.

PREPARATION OF FERMENTATION SUBSTRATES

Fresh litchi fruits procured from local market of Tezpur, Assam, India were washed, peeled, deseeded and transformed into pulp-free juice in a laboratory juice extractor (Philips, India). The juice was pasteurized at 90 ^{o}C for 1 min with constant stirring.

INOCULATION OF SUBSTRATES

Pasteurized juice in100 mL lots were taken into sterile Erlenmeyer flasks. Each flask containing 100 mL juice was inoculated with 1% culture each of Lp, Lr and La under aseptic conditions. No culture was added to the flask labeled as control. The flasks were then incubated at 37 ^{o}C. After 72 h of fermentation at 37 °C, the flasks were stored at 4 °C for 4 weeks. At an interval of 7 days, 10 mL of juice was taken out from each flask and tested for biochemical parameters viz. pH, acidity and sugar concentration and color change. A control (non fermented juice) containing sodium azide (1% w/v) was also evaluated throughout the storage stability study.

ENUMERATION OF BACTERIA

The enumeration of free probiotic cells was performed using method described by Shah and Lankaputhra (1997) and expressed in CFU/mL (colony forming unit). Enumeration of the probiotic bacteria in fruit juice was performed on weekly basis over a period of 4 weeks, using MRS agar after incubation at 37 °C for 72 h under aerobic conditions.

pH, TITRATABLE ACIDITY AND SUGAR CONTENT

The pH of each juice sample was measured in a pH meter (Eutech, Germany) after proper calibration. Titratable acidity, expressed as g lactic acid/100 g was determined by titration against 0.1N NaOH using phenolphthalein as an end point indicator. The reducing sugar content was estimated in terms of glucose (mg/mL) by phenol sulphuric acid method, as described by Dubios*et al.* (1956). All experiments were performed in triplicate to determine mean and standard error.

COLOUR ANALYSIS

The colour of the fermented litchi juice was determined by a Hunter Color Lab Ultra Scan-Vis colorimeter (USA). The colorimeter was calibrated and measurements were made through a 0.375 inch port/ viewing area. The reflectance instruments determined three color parameters: lightness (*L*), redness (*a*), and yellowness (*b*). Numerical values of *L*, a and b were converted into ΔE (total colour difference) according to Eq. (1). The reference value was the juice at the beginning of storage (0 day).

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

TOTAL PHENOLOLIC CONTENT AND ANTIOXIDANT ACTIVITY

The phenolic content in the probiotic fruit juice was assessed using a modified version of the Folin-Ciocalteau assay (Slinkard&Singelton, 1977). For the analysis, 20 µL each of extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100 µL of Folin–Ciocalteau reagent, mixed well and within 8 min, 300 µL of sodium carbonate was added. The samples were vortexed immediately and the tubes were incubated in the dark for 30 min at 40°C. The absorbance was then measured at 765 nm in a UV-Vis spectrophotometer (Cecil, Aquarius7400). Gallic acid was used as the standard and the results are expressed in mg GAE/100 g.

The antioxidant activity of the probiotic fruit juice was determined as radical scavenging activity as measured by the inhibition rate of DPPH (2, 2-diphenyl-1-picryllhydrazyl) radical (Brand-Williams, 1995). Precisely, 100 µL of the juice was added to 1.4 mL DPPH radical methanolic solution (10^{-4} M). The absorbance at 517 nm was measured at 30 min against blank using UV-Vis Spectrophotometer (Cecil, Aquarius 7400, UK). The results were expressed in terms of radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = \frac{A_o - A_s}{A_o} \times 100$$

STATISTICAL ANALYSIS

All experiments were carried out in triplicate and each sample was analyzed in duplicate. The results are expressed as mean ± SD (standard deviation). The data were statistically analyzed by Duncan's multiple range tests at $p \leq 0.05$ significant levels.

RESULTS AND DISCUSSION

The survival of the probiotic bacteria inoculated into litchi juice is shown in Table 1. All the three species of lactic acid bacteria viz. *L. plantarum* (Lp), *L. rhamnosus*(Lr) and *L. acidophilus* (La) were found to be capable of growing well on sterilized litchi juice without nutrient supplementation. *L. plantarum* grew rapidly on litchi juice and reached nearly x10^8 CFU/mL after 48 h of fermentation at 37°C. Extension of time beyond 48 h did not result in a significant increase in viable cell count of lactic acid bacteria.

Changes in pH, titratable acidity and sugars are presented in Fig 1. There was sharp decline in pH of the litchi juice due to fermentation. The titratable acidity expressed as lactic acid increased significantly and there was nearly 2% increase in acidity after 72 h of fermentation at 37 °C. The sugar content of the juice also decreased gradually due to consumption by the bacteria during fermentation.

Table 2 illustrates the effect of cold storage on the viability of the *L. plantarum* (Lp), *L. rhamnosus* (Lr) and *L. acidophilus* (La) in fermented litchi juice stored at 4°C for four weeks. The viability of the counts of all the three strains was higher than $x10^6$ CFU/mL even after 4 weeks of cold storage. Even though the viable count of Lp, Lr and La decreased slightly during cold storage, cell viability remained between 4.2 x 10^7 and 3.5 x 10^7 after 4 weeks of cold storage. A probiotic food product should retain at least 10^6 CFU/mL for maximum health benefits (Shah, 2001). The study showed that fermented litchi juice used was able to maintain the viable count of all the three strains even after 4 weeks of storage. Several factors could affect the cell viability of the lactic acid culture in probiotic food products. The main factors for loss of viability of probiotic organism have been attributed to the decrease in pH of the medium and accumulation of organic acids as a result of growth and fermentation (Shah & Jelen 1990).

No significant change was observed in the pH and acidity of juice incorporated with Lp and Lr during cold storage up to 4 weeks but litchi juice fortified with La showed decrease in acid level after 2 weeks. This may be due to the conversion of sugar into organic acids during fermentation. From the quality point of view, Lp and Lr have shown to be more promising strains compared to La for long storage period. Similar result was also reported by Yoon at el, (2004) in probiotication of tomato juice.

Colour analysis results of fermented and control litchi juice are presented in Table 3. No significant change was observed during the juice storage. The fact can be affirmed by the values of *a* parameter which was consistent during the storage period. The colour parameter *b* of the fermented juice showed slight increase indicating increase in yellowness. However, this change did not cause a significant visual difference when compared to the control due to low ΔE values. During the fermentation period, the colour difference between the control and fermented juice increased up to72 h of fermentation and stabilized there up to end the storage period (4 weeks).

Figure 3 illustrates the change in total phenolic content (TPC) and radical scavenging activity (measured by the inhibition rate of DPPH radical) of the litchi juice during fermentation period and storage period up to 4 weeks. Initial TPC of the litchi juice without probiotics was 1.51mg GAE/100 g which gradually lowered to 1.33mg GAE/100g after 72 h of fermentation and reached 0.38 mg GAE/100 g after 4 weeks of cold storage. The fermented juices also showed similar trend during fermentation up to 72 h and cold storage period. By comparing the four graphs in the Fig. 3, it perceived that DPPH activity of the fermented juice by all the three strains was higher than the control. In addition, statistical analysis revealed that the samples fermented by *L. plantarum* (Lp) and *L. rhamnosus* (Lr) had a significantly higher antioxidant activity compared to *L. Acidophilus* (La). This may be because the phenolic compounds found in fresh fruit juice are generally glycosylated with sugar that on fermentation of the juice and sugar consumption by micro organism undergo deglycosylation and release of the free hydroxyl groups and relevant aglycones which can contribute to the improved functional properties of the litchi juice.

Table 1 Enumeration of three stains of Lactobacillus in litchi juice during fermentation at 37 °C for 72 h

Time (h)	CFU/mL		
	Lp	Lr	La
0	$13.2 \pm 4.2 \times 10^{4\,a}$	$10.3 \pm 3.8 \times 10^{4\,a}$	$6.7 \pm 1.6 \times 10^{4\,a}$
24	$25.4 \pm 1.3 \times 10^{5\,b}$	$17.2 \pm 2.9 \times 10^{5\,b}$	$11.4 \pm 1.1 \times 10^{5\,b}$
48	$7.5 \pm 0.8 \times 10^{8\,c}$	$9.1 \pm 1.4 \times 10^{6\,c}$	$5.9 \pm 2.2 \times 10^{7\,c}$
72	$8.2 \pm 0.2 \times 10^{8\,c}$	$7.9 \pm 0.5 \times 10^{8\,d}$	$3.2 \pm 0.2 \times 10^{8\,d}$

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$); *L. plantarum* (Lp), *L. rhamnosus* (Lr) and *L. acidophilus* (La)

Table 2 Enumeration of three stains of Lactobacillus in fermented litchi juice during storage at 4°C

Time (week)	CFU/mL		
	Lp	Lr	La
1	$6.4 \pm 1.3 \times 10^{8\,a}$	$5.8 \pm 0.9 \times 10^{8\,a}$	$1.4 \pm 0.3 \times 10^{8\,a}$
2	$2.5 \pm 0.8 \times 10^{8\,a}$	$3.5 \pm 0.6 \times 10^{8\,b}$	$8.5 \pm 0.2 \times 10^{7\,b}$
3	$8.2 \pm 0.4 \times 10^{7\,b}$	$9.5 \pm 0.5 \times 10^{7\,c}$	$10.6 \pm 0.4 \times 10^{6\,c}$
4	$4.2 \pm 2.2 \times 10^{7\,c}$	$3.5 \pm 0.6 \times 10^{7\,c}$	$4.2 \pm 0.8 \times 10^{6\,d}$

Mean and standard deviation for n = 3. Means with different letters within the same row are statistically significant ($p<0.05$); *L. plantarum* (Lp), *L. rhamnosus* (Lr) and *L. acidophilus* (La)

Table 3 Colour change during fermentation and cold storage of the fermented litchi juice

Time	Litchi juice												
	L. plantarum					*L. rhamnosus*					*L. acidophilus*			
	L	*a*	*b*	*dE*		*L*	*a*	*b*	*dE*		*L*	*a*	*b*	*dE*
Control	10.61	6.87	6.97			10.61	6.87	6.97			10.64	6.89	6.99	
Fermented														

0 h	10.67	6.62	6.86	0.28		10.69	6.85	6.95	0.08		10.72	6.87	6.97	0.09
24 h	10.78	6.99	7.08	0.24		10.89	7.06	7.12	0.38		10.92	7.08	7.14	0.43
48 h	10.87	7.04	7.11	0.34		10.85	7.07	7.13	0.35		10.88	7.09	7.15	0.39
72 h	10.5	6.27	6.76	0.64		10.77	6.77	7.08	0.22		10.80	6.79	7.10	0.25
Stored														
1 week	11.02	6.96	7.23	0.5		10.88	6.79	7.1	0.31		10.91	6.81	7.12	0.35
2 week	10.64	6.73	6.97	0.14		10.73	6.78	7.03	0.16		10.76	6.80	7.05	0.18
3 week	11.15	6.97	7.76	0.55		11.17	6.46	7.41	0.32		11.20	6.48	7.43	0.36
4 week	11.22	7.06	7.43	0.38		11.56	6.08	7.59	0.21		11.59	6.10	7.61	0.24

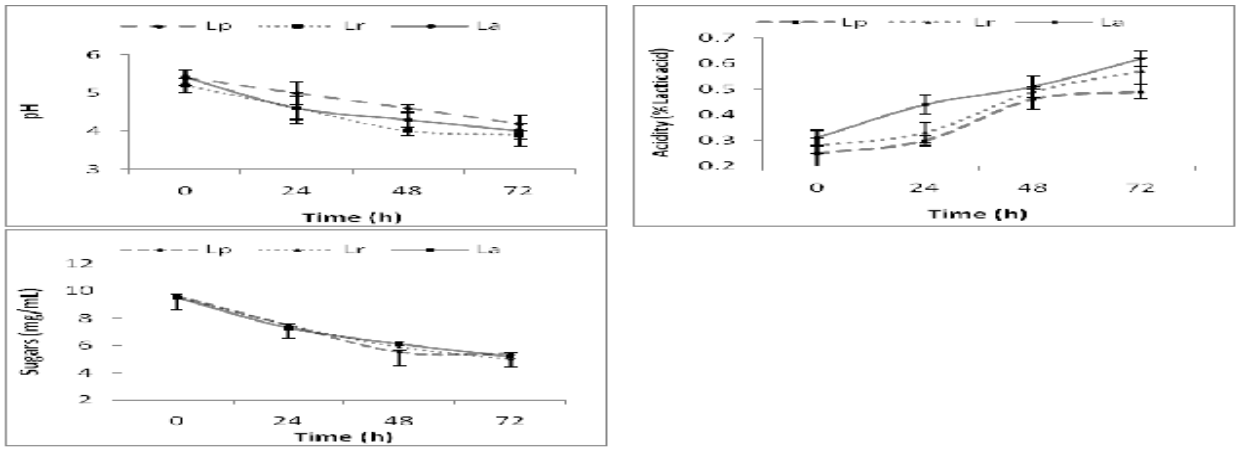

Figure 1 Changes in pH, acidity and sugars during lactic acid fermentation of litchi juice by *L. plantarum* (Lp), *L. rhamnosus* (Lr) and *L. acidophilus* (La)

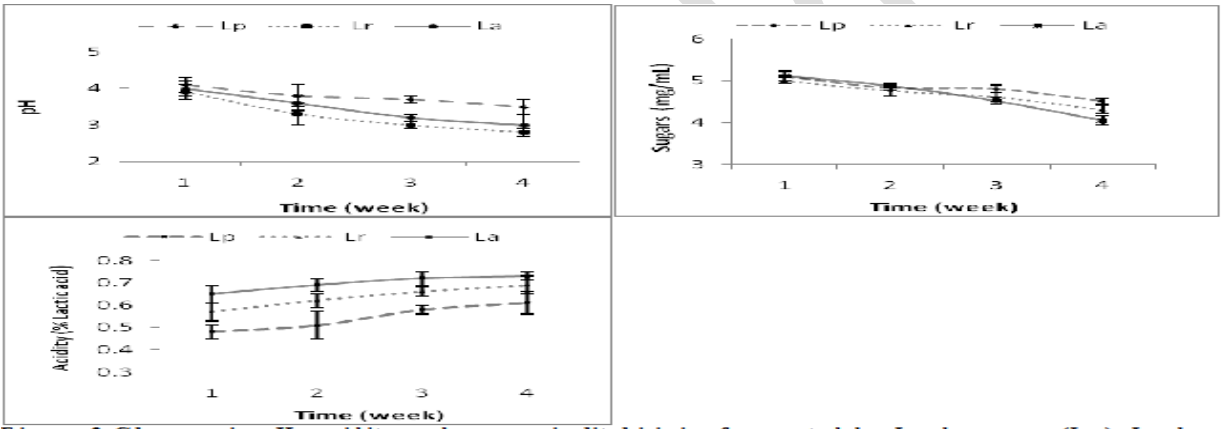

Figure 2 Changes in pH, acidity and sugars in litchi juice fermented by *L. plantarum* (Lp), *L. rhamnosus* (Lr) and *L. acidophilus* (La) stored at 4°C.

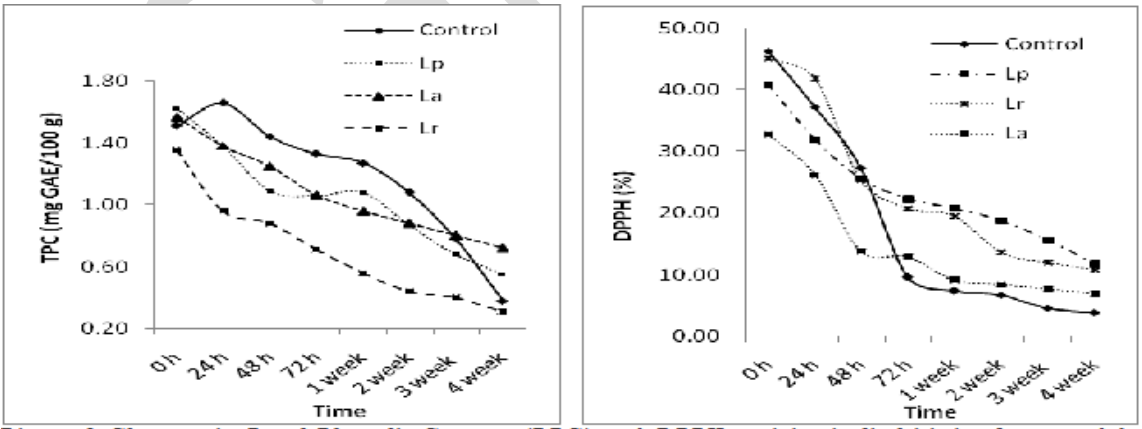

Figure 3 Changes in Total Phenolic Content (TPC) and DPPH activity in litchi juice fermented by *L. plantarum* (Lp), *L. rhamnosus* (Lr) and *L. acidophilus* (La) stored at 4°C.

CONCLUSION

The results of the investigation showed that *L. plantarum* (Lp) and *L. rhamnosus* (Lr) are capable of growing in litchi. These strains can metabolize sugar, organic acids and phenolic compounds. Fructose and glucose were utilized significantly as energy source by all the three strains of LAB. The color of the fermented juice was also found to be stable during cold storage up to one month. The free radical scavenging activity of the juice was enhanced fermentation; however enhanced effect of fermentation varied with employed bacteria since*L. plantarum* (Lp) and *L. rhamnosus* (Lr) exhibited greater improvement in the antioxidant activity of the fruit juice. Improvement of the free radical scavenging activity can be related to the production of other by-products through fermentation. These findings highlight the beneficial effect of litchi juice fermented by the incorporation of probiotic bacteria.

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