Development of antioxidant rich fruit supplemented probiotic yogurts using free and microencapsulated *Lactobacillus rhamnosus* culture

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Abstract The present study reports the preparation of probiotic yogurt using *Lactobacillus rhamnosus*. The standard starter cultures used for yogurt fermentation were *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and obtained from NDRI, Karnal, India. The prepared yogurt was supplemented with fruit pulp (10 % w/v) of apricot, raspberries, plum and jamun. These fruits were rich in antioxidant property as observed by DPPH, nitric oxide radical scavenging and ferric reducing antioxidant power assay methods. The probiotic yogurt products were prepared using free, alginate (2 %) and carrageenan (2 %) encapsulated probiotic *L. rhamnosus* culture. The microencapsulated beads were characterized by FTIR and alginate beads with bacteria showed characteristic wavelength major at 1424 and 1033 nm. The acidity increased (0.40 ± 0.07 – 0.76 ± 0.01 %) and pH of yogurts decreased (4.63 ± 0.06 – 2.83 ± 0.03) during storage. Probiotic *L. rhamnosus* count decreased during storage and alginate microencapsulated probiotic culture was more stable (8.85 ± 0.01 – 4.35 ± 0.03 log CFU/g) as compared with carrageenan encapsulated (8.79 ± 0.01 – 2.56 ± 0.04 log CFU/g) and free culture (8.90 ± 0.01 – 2.26 ± 0.03 log CFU/g). The antioxidant power of fruits

supplemented probiotic yogurts decreased successively during storage up to 15 days.

Keywords Probiotic *Lactobacillus rhamnosus* • Microencapsulation • FTIR • Antioxidant analysis • Fruit supplemented yogurt

Introduction

Microbial fermentation of dairy products is generally recognized for their beneficial effects on human health (Conly and Johnston 2004). Probiotics are live microorganisms that when administered in sufficient amounts confer a health benefit on the host (FAO/WHO 2001). The addition of probiotic cultures into diet improves digestion, nutrient absorption and promotes food safety (Mensah 1997; Duggan et al. 2002) and foods enriched with probiotics, prebiotics and synbiotics are recognized as functional foods.

Milk or milk products provide an excellent carrier for the probiotic organisms and the majority of probiotics are incorporated into milk powders, yogurt, cheese and ice cream (Dinakar and Mistry 1994; Desmond et al. 2002). Yogurt is prepared by fermentation of milk using standard *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* bacteria (FAO/WHO 1977) and probiotic yogurt contains probiotic culture in addition to standard cultures. There has been increase in trend to fortify the dairy product with fruits and fruit parts (Ghadge et al. 2008) to improve their nutritional value and the taste (Kailasapathy et al. 2008). Reactive oxygen species (ROS) produced in body during normal metabolism causes oxidative damage to DNA and protein (Halliwell 1997) and these ROS are responsible for the cardiovascular diseases and cancer (Parmar et al. 2010). Wild fruits viz. raspberries (*Rubus ellipticus*), plum (*Prunus domestica*), apricot (*Prunus*

Research highlights • Paper reports preparation of probiotic yogurts with and without fruit supplements

- Antioxidant activity of fruits was analyzed before and after addition in yogurts
- Free and microencapsulated probiotic *L. rhamnosus* was used for yogurt development
- Microencapsulation enhances the survival of probiotic culture in the yogurts

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armeniaca) and jamun (*Syzygium cumini*) are generally rich in antioxidants (Pantelidis et al. 2007; Ahn et al. 2007; Leccese et al. 2011; Belapurkar and Goel 2014). Antioxidants present in fruits can donate their electrons to ROS and neutralize their adverse effects (Kohen and Nyska 2002).

To confer health benefit, probiotics bacteria must arrive in intestines alive in sufficient numbers at 6–7 log CFU/g of products (Fuller 1989; Kailasapathy and Chin 2000; Krasaekoopt et al. 2003). The variation in viability of the probiotic cells is a major problem in the probiotic products. The viability of microbial cells can be improved by entrapping the cells by microencapsulation to produce particles with a diameter of a few nanometers to a few millimeters. Microencapsulation of probiotics with alginate or other gels generally improves the survival of probiotics in food products (Krasaekoopt et al. 2003, 2006; Crittenden et al. 2006; Heidebach et al. 2010). Keeping in view the potential of fruit antioxidants, the present study is endeavoured to develop antioxidant rich fruit supplemented probiotic yogurts with free and microencapsulated probiotic *Lactobacillus rhamnosus* culture, isolated earlier and reported in our previous publications (Kumar and Kumar 2014, 2015).

Material and methods

Bacterial strains

Standard yogurt cultures viz. *Streptococcus thermophilus* (NCDC 303) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (NCDC 253) were procured from National Dairy Research Institute, Karnal, India. The probiotic *Lactobacillus rhamnosus* culture isolated and characterized in our lab and as reported earlier in our publications (Kumar and Kumar 2014, 2015) was used in the present study.

Microencapsulation of probiotic *Lactobacillus rhamnosus*

Microencapsulation of the *Lactobacillus rhamnosus* was done according to the method as described by Vodnar et al. (2010) with some modifications. Alginate (2 % w/v) and carrageenan powder (2 % w/v) (HiMedia, India) were dissolved in sterile water. Briefly, 120 ml from each alginate and carrageenan solutions were mixed with 30 ml of bacterial suspension containing 7–8 log CFU/ml. The alginate emulsion was passed through the bioencapsulator (Buchi-390, India) nozzle (0.3 mm). However, the carrageenan emulsion was passed through syringe needle (0.5 mm) as carrageenan tends to solidify at 45 °C, and block the bioencapsulator nozzle. The emulsions of alginate and carrageenan were dropped into sterile solution of CaCl₂ (2 % w/v) and KCl (2 % w/v) respectively for stabilization of microencapsulated beads. The alginate and carrageenan beads were separated from the hardening solution

after 30 min by filtration and washed thrice with distilled water and were investigated further.

Fourier transform infrared (FTIR) spectroscopy analysis

The FTIR spectroscopy study was done using Cary 630, FTIR (Agilent Technologies, India) in the spectrum range from 4000 to 400 cm⁻¹ (Vodnar et al. 2010). The FTIR spectra were recorded separately for free bacterial suspension, free beads (alginate and carrageenan) and beads with bacteria.

Viability count of microencapsulated *Lactobacillus rhamnosus*

Briefly, 1 g of each bead type was added in 99 ml sodium citrate 1 % (w/v) at pH 6.0 and stirred continuously for 20 min at 150 rpm. The bacteria released from beads were counted on MRS agar plates at 37 °C after 48 h (Vodnar et al. 2010).

Antioxidant analysis of fruits used in yogurt supplementation

Selected wild underutilized fruits viz. apricot (*Prunus armeniaca*), raspberries (*Rubus ellipticus*), damson plum (*Prunus domestica*) were collected during May–September from Solan in Himachal Pradesh and jamun (*Syzygium cumini*) fruits were collected from Hamirpur in Himachal Pradesh, India and processed for pulp formation and pasteurized at 62.8 °C for 30 min. Antioxidant analysis viz. 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay, ferric reducing antioxidant power (FRAP) assay and nitric oxide radical scavenging (NORS) assay were performed with the aqueous solution of above selected fruits by dissolving 100 mg/100 ml (w/v) fruits in distilled water followed by sonication for 30 min at 25 °C. Ascorbic acid (20–100 µg/ml) was used as a standard.

2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging activity of the selected fruits was estimated according to the method described by Naznin and Hasan (2009). Briefly, from stock solution (200–1000 µg/ml sample) 2, 4, 6, 8 and 10 ml were taken in five test tubes and after this 2 ml of freshly prepared DPPH solution (0.004 % w/v in 95 % ethanol) was added to each test tube. The reaction mixture was incubated in the dark for 25 min and optical density was recorded at 523 nm against the blank (distilled water with sample). For control, 2 ml of DPPH solution was mixed with 10 ml of ethanol and optical density of the solution was recorded after 25 min. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity. Radical-scavenging

potential was expressed as IC₅₀ value, which represents the concentration scavenging 50 % of the DPPH radicals.

The DPPH radical scavenging was calculated using the following equation (Koleva et al. 2002):

$$\text{DPPH Scavenged (\%)} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

Ferric reducing antioxidant power (FRAP) assay

FRAP assay of the fruit samples was performed according to the method described by Oyaizu (1986). Briefly, 1 ml of test sample in different concentrations (200–1000 µg/ml) was mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and to this 1 ml of 1 % potassium ferricyanide was added. The reaction mixtures were incubated in a water bath at 50 °C for 20 min. After this, 1 ml of 10 % trichloroacetic acid was added to all test tubes and the mixtures were then centrifuged for 10 min at 5000 rpm at room temperature. Further, to 1 ml of each supernatant 1 ml of deionised water and 200 µl of 0.1 % FeCl₃ were added. The blank was prepared in similar way except that 1 % potassium ferricyanide was replaced by distilled water. The absorbance of all mixtures was recorded at 700 nm in spectrophotometer (EI, India). The reducing power was expressed as an increase in A₇₀₀ after blank subtraction (Banerjee et al. 2008).

Nitric oxide radical scavenging (NORS) assay

Nitric oxide radical scavenging activity was determined according to the method described by Garrat (1964) with modifications. Briefly, 2 ml of 10 M sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.2) was mixed with 1 ml of different concentrations (200–1000 µg/ml) of selected fruits samples and incubated at 25 °C for 2 h. From this mixture 1 ml was taken and to this 1 ml sulfanilic acid reagent was added (33 % in 20 % glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1 ml naphthylethylenediamine dihydrochloride (0.1 % w/v) was mixed and again incubated at room temperature for 30 min and the absorbance was recorded at 540 nm. The nitric oxide radicals scavenging activity was calculated using the same equation as mentioned earlier in DPPH radical scavenging assay.

Preparation of antioxidant rich fruit supplemented probiotic yogurts

Yogurt samples were prepared from cow milk according to the method described by Cakmakci et al. (2012) with modifications. Different types of yogurt viz. probiotic yogurt without fruits and selected fruit supplemented yogurt using free and microencapsulated probiotic culture were prepared in this study. Briefly, milk was analyzed for fats and solid not fat

(SNF) using Milkana KAM98-2A milk analyzer (Ekomilk ultra, India). Milk was heated to 80–85 °C for 20 min and cooled to 37–40 °C. Milk was divided in to six (1–6) parts (First part containing 98 ml and 2–5 parts containing 95 ml milk each) and the yogurt cultures (1:1 v/v) were added to all the parts. The first part was left unaltered, however, free (3 % v/v) probiotic *Lactobacillus rhamnosus* culture was added to other five parts and incubated at 37 °C for 18 h. Now, different fruit supplements viz. jamun, apricot, plum and raspberries (10 % w/v) were added to 2–5 parts and sixth part was left unaltered (probiotic yogurt without fruit supplements). All products were stored at 4 °C. Similarly, yogurts using alginate and carrageenan microencapsulated probiotic culture (3 % w/v) were also prepared.

Storage stability study of the yogurts

All yogurt types were analyzed at an interval of 5 days up to 15 days for acidity, pH, microbiological and antioxidants using following methodology to evaluate their storage stability.

pH, Acidity and selective enumeration of probiotic Lactobacillus rhamnosus in yogurts

The pH value of all yogurts during storage was recorded with digital pH meter (Deluxe pH meter, India). The titratable acidity (% lactic acid) was determined after mixing yogurts with 10 ml of distilled water and titrating with 0.1 N NaOH using 0.5 % phenolphthalein as indicator (AOAC 1990). Selective enumeration of probiotic in all product combinations during storage was performed according to the method described by Saccaro et al. (2011). MRS-vancomycin hydrochloride (MRS-V, pH 6.2) agar media was used for the selective enumeration of probiotic culture. For this, vancomycin (HiMedia, India) stock solution (100 mg in 5 ml w/v) was prepared and filtered (Millipore filter 0.2 µm) and 50 µl of this stock solution was added to 100 ml of sterile MRS agar at 40 °C just before pouring the plates. CFU/g was calculated using standard serial dilution method (Aneja 2006). Briefly, serial dilutions of 1 g of yogurt were prepared in peptone water for free probiotic culture and for microencapsulated culture the serial dilutions were prepared in sodium citrate and then 100 µl samples from different dilutions were spread over the solidified MRS-V medium and incubated at 37 °C for 48 h.

Antioxidant analysis of finished products during storage at 4 °C

Antioxidant analysis of the fruits supplemented yogurts was performed as discussed earlier. Briefly, 4 % (w/v) of antioxidant rich fruit supplemented yogurts were used for the antioxidant analysis. Probiotic yogurt without fruit supplements was used as negative control.

Statistical analysis

Analysis of variance (ANOVA) as well as average and standard error were determined using GraphPad Prism 6.0. A p value of <0.05 was considered to be statistically significant.

Results

Microencapsulation of *Lactobacillus rhamnosus*, FTIR spectroscopy and viability count of the microcapsules

Probiotic *Lactobacillus rhamnosus* culture was microencapsulated in alginate and carrageenan matrix to evaluate the survival of probiotic culture in the yogurt. Both alginate and carrageenan beads were round in shape and the alginate beads prepared using Bioencapsulator were 0.3 mm in size and carrageenan beads size ranged from

0.5 to 1 mm. The comparative FTIR spectrum of encapsulated bacteria in alginate and carrageenan beads versus free bacteria and free beads was obtained in the spectrum range of $4000\text{--}400\text{ cm}^{-1}$ (Fig. 1). The intensity of the peak increased when bacteria were encapsulated in the alginate and carrageenan. Four regions were identified and marked as 1–4 (Fig. 1). The region 1 is located in between 3600 and 3100 cm^{-1} which attributes to OH stretching. The region 2 is located between 1730 and 1625 cm^{-1} and is associated with C=O stretching vibrations of esterified lipids and fatty acids as suggested by Kansiz et al. (1999). The region 3 is located in $1557\text{--}1412\text{ cm}^{-1}$ which is attributed to COO stretching. The region 4 is located in between 1300 and 900 cm^{-1} and attributes to COC stretching vibrations of carbohydrate scaffolds of alginate or carrageenan as suggested by Kansiz et al. (1999). The entrapment of bacteria in alginate and carrageenan beads containing $7\text{--}8\text{ log CFU/g}$ was also confirmed by colony count method.

Fig. 1 Comparative FTIR spectra of free *L. rhamnosus*, free beads and beads with *L. rhamnosus* in (a) alginate and (b) carrageenan. Four regions (1, 2, 3 and 4) discriminate the presence of bacteria in the beads in spectrum range $4000\text{--}400\text{ cm}^{-1}$

Antioxidant analysis of fruits used in yogurt preparation

All selected fruits were analyzed for antioxidant potential before supplementation in products. In DPPH radical scavenging assay the percentage scavenging increased (46.13 ± 0.78 – 71.76 ± 0.45 %) with increase in the concentration (200–1000 $\mu\text{g/ml}$ w/v) of the fruit supplements. IC_{50} value of all the selected fruits was observed in the concentration range of 200–400 $\mu\text{g/ml}$. However, in jamun fruit IC_{50} value was <200 $\mu\text{g/ml}$ (Fig. 2a). In FRAP assay a linear increase (0.391 ± 0.01 – 0.698 ± 0.02 %) in reducing power of all fruit samples was reported with increase in concentration range of 200–1000 $\mu\text{g/ml}$ (w/v) (Fig. 2b). Similarly, with increase in concentration (200–1000 $\mu\text{g/ml}$ w/v) of the fruits there is significant increase (44.27 ± 0.75 – 77.47 ± 1.01 %) in the percentage scavenging of nitric oxide radical (Fig. 2c). IC_{50} value of all selected fruits was observed in the concentration range of 200–400 $\mu\text{g/ml}$ except with apricot fruit where IC_{50} value was reported in 400–600 $\mu\text{g/ml}$.

Storage stability study of probiotic fruit supplemented yogurts

Different yogurts were prepared from cow milk containing 4.6 ± 0 % fat and 8.87 ± 0.06 % SNF. The pH of the all yogurt

types decreased and acidity increased up to 15 days during storage at 4 °C (Table 1). The antioxidant rich fruit supplemented probiotic yogurt showed low pH values as compared to control probiotic yogurt without fruits with high pH. This decrease in pH is attributed due to the acidity of the fruits.

To confer health benefit, probiotic bacteria must arrive in intestine alive and in sufficient numbers i.e. 6–7 log CFU/g of product. The MRS-V medium was used for selective enumeration of *L. rhamnosus* as this medium did not favor the growth of standard yogurt cultures. There is significant reduction in the number of viable cells inevitably due to the processing conditions especially low pH and high acidity during storage. The yogurts developed using free probiotic culture retained probiotic values of 6–7 log CFU/g up to 5 days of storage at 4 °C. However, control (yogurt without fruit supplements) and jamun supplemented yogurt retained this value up to 10 days of storage.

All the yogurts prepared using alginate microencapsulated probiotic culture retained probiotic values of 6–7 log CFU/g up to 10 days of storage at 4 °C. The yogurts prepared using carrageenan encapsulated probiotic retained probiotic value up to 5 days of storage at 4 °C, however the control yogurt and jamun supplemented yogurt retained the probiotic value up to 10 days of storage. Alginate and carrageenan microencapsulated probiotic culture were more stable in the yogurts as

Fig. 2 Antioxidant analysis of selected fruit supplements before addition in the yogurts. (a) DPPH radical scavenging assay, (b) Ferric reducing antioxidant power (FRAP) assay, (c) Nitric oxide radical scavenging (NORS) assay

Table 1 Physicochemical and microbiological analysis of different types of yogurt prepared using free, alginate and carrageenan encapsulated probiotic *Lactobacillus rhamnosus* culture during storage at 4 °C

Yogurt types	Storage time (days)	Yogurt prepared using free probiotic culture			Yogurt prepared using alginate encapsulated probiotic culture			Yogurt prepared using carrageenan encapsulated probiotic culture		
		pH	Acidity (%)	log CFU/g	pH	Acidity (%)	log CFU/g	pH	Acidity (%)	log CFU/g
Control without fruit supplement	1	4.49±0.08 a	0.45±0.01 a	8.90±0.01 a	4.63±0.06 a	0.40±0 a	8.80±0.01 a	4.45±0.10 a	0.46±0.01 a	8.79±0.01 a
	5	4.24±0.06 b	0.51±0.01 a	8.87±0.03 b	4.46±0.09 b	0.46±0.01 a	8.69±0.02 b	4.22±0.06 b	0.52±0.01 a	8.30±0.02 b
	10	3.97±0.08 c	0.55±0.01 b	6.47±0.02 c	4.18±0.08 c	0.52±0.01 b	7.52±0.02 c	4.00±0.02 c	0.54±0 a	7.47±0.02 c
	15	3.8±0.08 d	0.58±0.01 c	4.04±0.04 d	3.99±0.02 d	0.54±0.01 c	6.42±0.04 d	2.91±0.07 d	0.74±0.02 b	5.28±0.05 d
Jamon supplemented	1	4.17±0.05 a	0.53±0.01 a	8.87±0.01 a	4.09±0.09 a	0.53±0.02 a	8.85±0.01 a	3.96±0.09 a	0.55±0.01 a	8.76±0.02 a
	5	3.92±0.1 b	0.55±0.01 a	7.59±0.02 b	3.81±0.08 b	0.58±0.01 a	8.48±0.02 b	3.76±0.06 b	0.59±0.01 a	8.04±0.04 b
	10	3.82±0.04 c	0.58±0 a	6.40±0.04 c	3.58±0.01 c	0.63±0 b	7.56±0.02 c	3.50±0.01 c	0.62±0 a	7.35±0.03 c
	15	3.49±0.07 d	0.62±0.01 b	3.47±0.02 d	3.19±0.07 d	0.72±0.01 c	6.1±0.07 d	3.05±0.09 d	0.72±0.01 b	3.59±0.01 d
Apricot supplemented	1	4.08±0.08 a	0.54±0.01 a	8.79±0.01 a	4.11±0.08 a	0.53±0.01 a	8.81±0.02 a	4.08±0.12 a	0.53±0.02 a	8.78±0.02 a
	5	3.73±0.12 b	0.58±0.01 a	6.51±0.03 b	3.72±0.09 b	0.59±0.01 a	7.66±0.03 b	3.65±0.07 b	0.60±0.01 a	7.59±0.02 b
	10	3.51±0.05 c	0.61±0 a	5.38±0.03 c	3.23±0.09 c	0.68±0.01 b	6.56±0.02 c	3.31±0.09 c	0.69±0.01 b	6.03±0.02 c
	15	3.32±0.05 d	0.68±0.01 b	2.58±0.02 d	3±0.01 d	0.72±0.01 c	5.47±0.02 d	2.94±0.07 d	0.73±0.01 c	3.36±0.02 d
Raspberries supplemented	1	3.90±0.08 a	0.53±0.01 a	8.84±0.01 a	3.91±0.07 a	0.53±0.01 a	8.80±0.01 a	3.93±0.11 a	0.55±0.02 a	8.73±0.01 a
	5	3.71±0.08 b	0.58±0.01 a	6.48±0.03 b	3.63±0.05 b	0.61±0.01 b	7.52±0.01 b	3.72±0.12 a	0.60±0.01 a	6.70±0.01 b
	10	3.57±0.08 c	0.62±0 a	4.63±0.03 c	3.25±0.03 c	0.68±0.01 c	6.05±0.06 c	3.29±0.10 b	0.67±0.02 a	5.56±0.02 c
	15	3.13±0.14 d	0.70±0.02 b	2.26±0.03 d	2.83±0.03 d	0.76±0.01 d	4.35±0.03 d	2.94±0.05 c	0.73±0.01 a	2.56±0.04 d
Plum supplemented	1	3.90±0.04 a	0.56±0.01 a	8.85±0.01 a	3.94±0.06 a	0.55±0.01 a	8.78±0.02 a	3.94±0.07 a	0.57±0.02 a	8.76±0.01 a
	5	3.71±0.05 b	0.59±0.01 a	6.53±0.01 b	3.52±0.09 b	0.65±0.02 b	7.68±0.01 b	3.54±0.06 b	0.63±0.02 a	6.63±0.01 b
	10	3.57±0.02 c	0.62±0.01 a	5.61±0.02 c	3.30±0.03 c	0.69±0.01 c	6.42±0.02 c	3.28±0.12 c	0.68±0.01 a	5.62±0.02 c
	15	3.13±0.12 d	0.71±0.01 b	3.47±0.04 d	3.11±0.09 d	0.71±0.01 d	5.46±0.01 d	3.06±0.06 d	0.72±0 a	3.55±0.02 d

When mean values were significantly different ($P<0.05$), different letters were applied (a, b, c, d)

Fig. 3 Antioxidant (DPPH, FRAP, NORS) analysis of fruits supplemented probiotic yogurt developed using 1) free probiotic *L. rhamnosus* culture, 2) alginate encapsulated probiotic culture, 3) and carrageenan encapsulated probiotic culture on 1, 5, 10 and 15 days of

storage at 4 °C. P-Plum, A-Apricot, R-Raspberries and J-Jamun. When mean values were significantly different ($P < 0.05$), different letters were applied (a, b, c, d)

compared to free probiotic culture. Further alginate microencapsulated probiotic culture was more stable as compared with carrageenan encapsulated culture (Table 1). Fruit supplemented yogurts (4 %w/v) were used for the antioxidant analysis in the present study and the results are shown in Fig. 3. The significant decrease in the antioxidant activity of the fruit supplemented probiotic yogurts was reported during storage. In DPPH radical scavenging and NORS assay the percentage scavenging decreased from 69.32 ± 0.25 – 49.12 ± 0.76 and 64.91 ± 0.78 – 49.56 ± 1.28 respectively. In FRAP assay the optical density decreased from 0.649 ± 0 to 0.478 ± 0 during storage up to 15 days.

Discussion

In the present study probiotic yogurts and antioxidant rich fruit supplemented yogurts were prepared and investigated for their storage stability. The probiotic *Lactobacillus rhamnosus* culture was added in the yogurts in two forms viz. as free culture and as microencapsulated culture. FTIR analysis was performed and the intensity of peak increased in beads with bacteria which is evident for entrapment of bacteria in the beads. The entrapment of bacteria in the beads was also confirmed by the CFU count containing 7–8 log CFU/g. Vodnar et al. (2010) also reported FTIR fingerprint

of the free bacterial suspension, beads and beads with bacteria and results were comparable to the present findings.

Selected fruits used in present study were rich in antioxidants as evaluated by DPPH radical scavenging, FRAP and NORS assay. In DPPH and nitric oxide radical scavenging assay the percentage scavenging increased with the increase in concentration (200–1000 µg/ml w/v) of the selected fruit supplements respectively. However, in FRAP assay linear increase in reducing power was reported over concentration range of 200 – 1000 µg/ml (w/v) fruit supplements. Results of the present study were analyzed in light of the existing literature. A large number of wild fruits in Himachal Pradesh are underutilized and as a result of this a lot of natural produce goes as waste. Therefore, some wild fruits viz. apricot (*Prunus armeniaca*), raspberries (*Rubus ellipticus*), damson plum (*Prunus domestica*) and jamun (*Syzygium cumini*) were used for the development of fruit nutraceutical supplemented probiotic products. The antioxidant potential of these fruits was reported by a number of investigators (Pantelidis et al. 2007; Ahn et al. 2007; Leccese et al. 2011; Schmitzer et al. 2011; Bobinaite et al. 2012; Belapurkar and Goel 2014).

In this study pH of all yogurts decreased (4.63 ± 0.06 – 2.83 ± 0.03) and acidity increased (0.40 ± 0 – 0.76 ± 0.01) during 15 days of storage. Antioxidant rich fruit supplemented yogurts showed low pH values and increased acidity as compared with yogurts without fruit supplements (Table 1). Hossain et al. (2012) also developed fruit fortified yogurts and reported decrease in pH and increase in acidity in fruit supplemented yogurt and this change is attributed due to the acidity of fruits. Donkor et al. (2006) reported that *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* are responsible for the post acidification of yogurts during cold storage. Similar results were also reported by the others (Vinderola et al. 2000; Bakirci and Kavaz 2008).

A significant reduction in the number of viable probiotic cells in all yogurts was reported in the present study which is attributed to the processing conditions especially low pH and high acidity during storage. The variation in survival of the free probiotic cells is a major problem in the probiotic products, therefore, microencapsulated beads of the probiotic bacteria were used in the present study to evaluate their survival in the yogurts. The alginate microencapsulated probiotic culture was found more stable as compared with free probiotic and carrageenan encapsulated probiotic culture. Canganella et al. (1998) and Vahedi et al. (2008) also developed fruit yogurt formulations and observed decrease in lactobacilli count during storage. Krasaekoopt et al. (2003) reported the improved survival of probiotic microorganisms in the products when microencapsulated in alginate or other gels. Similar findings were reported by Krasaekoopt et al. (2006) and Crittenden et al. (2006).

The antioxidant activity in fruits supplemented yogurts decreased continuously over a storage period from 1 to 15 days

which may be attributed to denaturation during storage time and temperature (Fig. 3). Lawin and Kongbangkerd (2010) reported decrease in antioxidant activity of yogurts fortified with roselle syrup to nil after 6 days of storage due to loss of anthocyanin activity. El-Said et al. (2014) also developed stirred yogurt fortified with pomegranate peel extracts and evaluated the antioxidant activity of the yogurts.

Conclusion

In conclusion the results of present study supported *Lactobacillus rhamnosus* culture with potential for use in probiotic and antioxidant rich fruit nutraceutical supplemented probiotic yogurt preparations. The alginate microencapsulated probiotic culture was found more stable as compared to free probiotic and carrageenan encapsulated probiotic culture in the yogurts. The antioxidant activity of the antioxidant rich fruit supplemented yogurts decreased continuously but they retained useful amount of antioxidants up to 15 days of storage. However, detailed in vivo animal experiments or cell line studies and sensory studies will be required to prove the commercial acceptability of the products.

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