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Development of Wild Fruits Fortified Probiotic Milk using *Lactobacillus rhamnosus* Culture

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ABSTRACT: Probiotic milk samples were prepared using free, alginate (2%) and carrageenan (2%) encapsulated probiotic culture and fortified with fruit juice (10% v/v) of *Prunus armeniaca, Rubus ellipticus, Prunus domestica* and *Syzygium cumini.* The pH of milk samples decreased (6.06±0.06–3.33±0.08) and acidity increased (0.18±0–0.67±0.02 %) during 15 days of storage. Alginate microencapsulated culture was more stable as compared with carrageenan encapsulated and free culture. Milk samples prepared using alginate microencapsulated culture retained probiotic values of 6.65±0.02 and 6.55±0.03 log CFU/mL respectively in *Syzygium cumini* and *Prunus domestica* fortified probiotic milk up to day 5 of storage. The antioxidant power of fruit fortified probiotic milk decreased during storage. In DPPH radical scavenging and NORS assay percentage scavenging decreased from 61.09±1.17 to 38.77±1.42 and 62.66±0.56 to 40.61±0.90 respectively. In FRAP assay optical density decreased from 0.598±0.01 to 0.366±0.02 during storage up to 15 days.

Keywords: Probiotic *Lactobacillus rhamnosus,* microencapsulation, fruit fortified probiotic milk, antioxidant analysis

INTRODUCTION

Probiotic is a functional food which is essential for good health. The term probiotic was introduced in 1965 by Stillwell and Lilly. Probiotics are microorganisms that when administered in sufficient amount confer a health benefit on the host and are also called friendly bacteria (FAO/WHO, 2001). Milk and milk products provide an excellent carrier for the probiotic microorganisms and most of them can readily utilize lactose as an energy source for their growth. Milk protein also provides important protection to the probiotic bacteria during passage through stomach (Charteris *et al.,* 1998). There has been increase in trend to fortify the dairy product with fruits and fruit parts to improve their nutritional value and the taste (Ghadge *et al.,* 2008; Kailasapathy *et al.,* 2008).

Wild fruits viz. raspberries (*Rubus ellipticus*), plum (*Prunus domestica*), apricot (*Prunus armeniaca*) and jamun (*Syzygium cumini*) were found rich in antioxidants as reported in our previous publication (Kumar and Kumar, 2016). The free radicals are responsible for aging and several degenerative diseases e.g. heart disease, cataracts, cognitive dysfunction and cancer (Lyras *et al.,* 1997; Sayre *et al.,* 2001). The defense system that prevents the body from damage by the free radicals is called antioxidants (Kunwar and Priyadarsini, 2011). Probiotics bacteria must arrive in intestines alive and in sufficient numbers i.e. 6-7 log CFU/g of products to confer health beneficial effects. The probiotic products face the problem of variation in viability of cultures in various developed products and this can be improved by using microencapsulated probiotic culture. Microencapsulation of probiotic microorganisms with alginate or other gels generally improves survival of probiotics in food products (Krasaekoopt *et al.,* 2003, 2006; Heidebach *et al.,* 2010; Kumar and Kumar, 2016). Keeping in view the importance of above work, the present study is endeavored to develop fruit fortified probiotic milk using *Lactobacillus rhamnosus* culture.

MATERIALS AND METHODS

A. Bacterial Strain

The probiotic *Lactobacillus rhamnosus* LBS 2 culture was isolated and characterized as reported in our previous publications (Kumar and Kumar, 2014, 2015).

B. Microencapsulation, FTIR spectroscopy analysis and viability count of L. rhamnosus

Microencapsulation of the *Lactobacillus rhamnosus* was performed using alginate (2% w/v) and carrageenan (2% w/v) as described by Kumar and Kumar (2016). The microencapsulated beads were characterized using FTIR spectroscopy. For viability count, 1 g of each bead type was dipped 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 (de Man, Rogosa, and Sharpe) agar (Vodnar *et al.*, 2010).

C. Antioxidant analysis of fruits selected for fortification of milk

Different antioxidant analysis viz. 2, 2-diphenyl-l-picryl hydrazyl (DPPH) radical scavenging assay (Naznin and Hasan, 2009), ferric reducing antioxidant power (FRAP) assay (Oyaizu, 1986) and nitric oxide radical scavenging (NORS) assay (Garrat, 1964) were performed with wild fruits viz. apricot (*Prunus armeniaca*), wild raspberries (*Rubus ellipticus*) and damson plum (*Prunus domestica*) and jamun (*Syzygium cumini*) and described by Kumar and Kumar (2016). Ascorbic acid (20–100 µg/mL) was used as a standard.

D. Preparation of fruit fortified probiotic milk

All probiotic milk samples were prepared from the cow milk. Briefly, milk sample was analyzed for fats and solid not fat (SNF) using Milkana KAM98-2A milk analyzer (Ekomilk ultra, India) and heated to 80-85ºC for 20 min and cooled to 37-40ºC. Three sets of probiotic milk viz. probiotic milk without fruits and fruits fortified milk were prepared using free, alginate and carrageenan encapsulated *L. rhamnosus* culture. In first set, milk was divided in to five parts (First part 95 mL and 2-5 parts were 85 mL each). Probiotic *Lactobacillus rhamnosus* LBS 2 culture (5% v/v containing 10^7-10^8 CFU/mL) was added to all parts. First part was left unaltered and pasteurized juice of selected fruits viz. jamun, apricot, plum and raspberries (10% v/v) was added to 2-5 parts followed by homogenization and storage at 4°C. In second set, probiotic milk with and without fruit fortification were prepared in similar way as described above by replacing the free culture with alginate encapsulated probiotic culture (5% w/v containing 10^7-10^8 CFU/g). In third set, probiotic milk samples were prepared using carrageenan encapsulated probiotic culture under similar conditions and stored at 4°C for further study.

E. Storage stability study of the finished probiotic milk products

All probiotic milk samples were analyzed for pH, acidity and probiotic cell count on 1, 5, 10 and 15 days of storage at 4ºC. The pH value of the milk samples during storage was recorded with digital pH meter (Deluxe pH meter, India). The titratable acidity (% lactic acid) was determined after mixing milk samples with 10 mL of distilled water and titrating with 0.1 N NaOH using 0.5% phenolphthalein as indicator (AOAC, 1990). Probiotic *L. rhamnosus* count was performed according to the method described by Saccaro *et al.* (2011). MRS -vancomycin hydrochloride (MRS-V) agar media was used for probiotic cell count as described in detail by Kumar and Kumar (2016).

F. Antioxidant analysis of finished milk products during storage

Antioxidant analysis of the fruits fortified milk was done during storage period (1-15 days) at an interval of 5 days. Briefly, 4% (v/v) of antioxidant rich fruit supplemented milk samples were used for the antioxidant analysis. Different antioxidant tests viz. DPPH radical scavenging assay, FRAP assay and NORS assay were performed as per the protocols described in detail in our previous publication (Kumar and Kumar, 2016). Probiotic milk without fruit supplements was used as a negative control.

G. Statistical analysis

Results were expressed as mean ± S.D. All data were analyzed by means of analysis of variance, average and standard error using Graph Pad Prism 5.0. A p value of < 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

A. Microencapsulation, FTIR spectroscopy and viability count of L. rhamnosus

Probiotic *L. rhamnosus* culture was microencapsulated in alginate and carrageenan matrix to evaluate its survival in milk products. The comparative FTIR spectrum of encapsulated bacteria in alginate and carrageenan beads, free bacteria and free beads was obtained. The intensity of the peak increased when bacteria were encapsulated in the alginate or carrageenan (Kumar and Kumar, 2016). The entrapment of bacteria in beads containing 7-8 log CFU/g was also confirmed by colony count method. Detailed results of microencapsulation and FTIR spectroscopy were reported in our previous publication (Kumar and Kumar, 2016).

B. Antioxidant analysis of selected fruits

All selected fruits were analyzed for antioxidant activity before fortification in probiotic milk. Detailed description of antioxidant analysis of selected fruits before their supplementation is reported earlier (Kumar and Kumar, 2016). Briefly, in DPPH radical scavenging assay IC_{50} value of all fruits was observed in the concentration range of 200-400 µg/mL except in jamun fruit where IC_{50} value was <200 µg/mL. In FRAP assay increase in reducing power (0.391±0.01-0.698±0.02%) of all fruits was reported with increase in concentration range of 200–1000 µg/mL (w/v) In NORS assay, IC_{50} value of all fruits was observed in the concentration range of 200-400 µg/mL except apricot fruit where IC_{50} value was reported in 400-600 µg/mL.

C. Storage stability of probiotic fruit fortified milk

Probiotic milk without fruits. The cow milk containing 4.3±0.01% fat and 8.57±0.08% SNF (Solid not fat) was used for the preparation of probiotic *L. rhamnosus* supplemented milk products. The pH of the all milk samples decreased (6.06±0.06–3.74±0.07) and acidity increased (0.18±0–0.58±0.02%) during storage at 4ºC up to 15 days (Table 1). Probiotic bacteria must arrive alive in intestine and in sufficient numbers i.e. 6-7 log CFU/g of product to confer health benefits. The milk samples prepared using free, alginate and carrageenan encapsulated probiotic culture retained probiotic values of 6.75±0.02 log CFU/mL, 6.61±0.03 log CFU/mL and 6.61±0.03 log CFU/mL respectively up to 5 days of storage at 4°C. No growth was observed on day 15 in probiotic milk prepared using free and carrageenan encapsulated probiotic culture. However, growth of 2.33±0.03 log CFU/mL was observed with alginate encapsulated *L. rhamnosus* even after 15 days of storage (Table 1). The reason for low viable count might be due to high initial pH (6.06±0.06) and abrupt high decrease in pH of the milk samples during storage. Amiri *et al.* (2010) developed acidophilius milk with probiotics and prebiotics using cultures of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, *Lactobacillus casei* and the product retained the probiotic value of 8-10 log CFU/mL.

Fruit fortified probiotic milk. The fruit fortified probiotic milk samples were prepared from cow milk containing 4.4±0.05% fat and 8.43±0.09% SNF. The pH of the all milk samples decreased (5.73±0.05–3.33±0.08) and acidity increased (0.23±0–0.67±0.02%) during storage at 4ºC up to 15 days (Table 1). There is continuous reduction in the number of viable probiotic cells during storage. The jamun, apricot, raspberries and plum supplemented milk samples prepared using free probiotic culture retained a probiotic value of 6.70±0.02, 7.14±0.09, 6.63±0.02 and 6.59±0.02 log CFU/mL respectively only on first day of storage and no growth was observed on day 15 during storage in all milk samples while in apricot and raspberries supplemented milk samples no growth was observed on day 10 of storage (Table 1). Milk samples prepared using alginate microencapsulated probiotic culture retained probiotic values of 6.65±0.02 and 6.55±0.03 log CFU/mL respectively in jamun and plum supplemented probiotic milk up to 5 days of storage at 4°C, whereas, the apricot and raspberries supplemented milk retained probiotic value of 7.04±0.04 and 6.61±0.01 respectively only on day 1 of storage. No growth was observed on day 15 during storage in raspberries, plum and jamun supplemented probiotic milk. However, apricot supplemented probiotic milk retained 2.32±0.02 log CFU/mL on day 15 during storage at 4ºC. The fruit supplemented probiotic milk samples prepared using carrageenan encapsulated culture retained probiotic value of 6.57±0.03 log CFU/mL up to 5 days of storage only in jamun supplemented probiotic milk, whereas, apricot, raspberries and plum supplemented probiotic milk samples retained probiotic value of 6.53±0.03, 6.59±0.02 and 7.06±0.10 log CFU/mL respectively only on day 1 of storage and after this decreased continuously. No growth was reported on day 15 during storage in all milk samples (Table 1).

The results obtained in the present study were compared in light with the existing literature. Junaid *et al.* (2013) developed strawberry, pineapple and mango flavored probiotic acidophilus milk using probiotic starter culture *Lactobacillus acidophilus* and studied its microbiological and physicochemical properties up to 6 days of storage. In this study a slight increase in acidity of the milk was observed after 6 days of storage resulting in a decrease of pH (from 4.5 to 4.3) and there was also slight decrease in the viability of cells. Mousavi *et al.* (2011) developed probiotic pomegranate juice through fermentation using four strains of lactic acid bacteria viz. *Lactobacillus plantarum*, *L. delbruekii*, *L. paracasei* and *L. acidophilus.* They observed that the viable cells remained at their maximum level up to 2 weeks and decreased sharply after 4 weeks of storage. Mohan *et al.* (2013) developed probiotic fruit juices by using *Lactobacillus acidophilus.* They reported decrease in pH and increase in acidity. The encapsulated probiotic culture was more stable as compared to free probiotic culture during storage.

D. Antioxidant analysis of finished products during storage

Fruit fortified milk samples (4% v/v) were used for the antioxidant analysis in the present study and the results are shown in Fig. 1.

Table 1: Physicochemical and microbiological analysis of milk samples prepared using free, alginate and carrageenan encapsulated probiotic *Lactobacillus rhamnosus* culture during storage at 4ºC.

Milk types	Storage time (days)	Milk prepared using free probiotic culture			Milk prepared using alginate (2 %) encapsulated probiotic culture			Milk prepared using carrageenan (2 %) encapsulated probiotic culture		
		pH[1)]	Acidity (%)[1)]	log CFU/mL[1)]	pH	Acidity (%)	log CFU/mL	pH	Acidity (%)	log CFU/mL
	1	6.01±0.02 a[2)]	0.18±0 a	7.28±0.04 a	6.06 ± 0.05 a	0.19 ± 0.01 a	6.99±0.09 a	6.06±0.06 a	0.19±0.02 a	7.06±0.08 a
Control	5	5.41±0.05 b	0.28±0.01 b	6.75±0.02 b	5.33±0.05 b	0.33±0.01 b	6.61±0.03 b	5.26±0.05 b	0.33±0.01 b	6.61±0.03 b
without fruit	10	4.88±0.08 c	0.37±0.01 c	3.51±0.03 c	4.73±0.10 c	0.41±0.02 c	3.56±0.02 c	4.78±0.05 c	0.41±0 c	3.73±0.03 c
fortification	15	3.75±0.06 d	0.58±0.02 d	ND[3)]	3.79±0.08 d	0.57±0.01 d	2.33±0.03 d	3.74±0.07 d	0.58±0.02 d	ND
	1	5.61±0.03 a	0.26±0.01 a	6.70±0.02 a	5.70±0.07 a	0.24±0.01 a	7.09±0.09 a	5.73±0.05 a	0.23±0 a	7.08±0.08 a
Jamun	5	4.96±0.05 b	0.36±0.01 b	4.79±0.02 b	4.88±0.08 b	0.40±0.01 b	6.65±0.02 b	5.14±0.06 b	0.35±0.01 b	6.57±0.03 b
fortified	10	4.58±0.06 c	0.45±0.01 c	2.54±0.02 c	4.55±0.06 c	0.45±0.01 c	4.54±0.03 c	4.72±0.03 c	0.43±0.02 c	2.48±0.03 c
	15	3.53±0.05 d	0.62±0.01 d	ND	3.73±0.11 d	0.59±0.02 d	ND	3.83±0.08 d	0.56±0.02 d	ND
	1	5.59±0.07 a	0.29±0.01 a	7.14±0.09 a	5.64±0.06 a	0.26±0.01 a	7.04±0.04 a	5.66±0.05 a	0.24±0.02 a	6.53±0.03 a
Apricot	5	4.80±0.06 b	0.41±0.01 b	4.59±0.02 b	5.03±0.05 b	0.38±0.02 b	5.57±0.03 b	4.92±0.04 b	0.39±0.01 b	5.44±0.03 b
fortified	10	4.37±0.05 c	0.50±0.01 c	ND	4.56±0.10 c	0.48±0.02 c	4.46±0.03 c	4.62±0.04 c	0.45±0.01 c	2.43±0.02 c
	15	3.49±0.08 d	0.63±0.02 d	ND	3.46±0.05 d	0.64±0.01 d	2.32±0.02 d	3.51±0.02 d	0.63±0.01 d	ND
Raspberries	1	5.56±0.06 a	0.28±0.01 a	6.63±0.02 a	5.53±0.06 a	0.28±0.02 a	6.61±0.01 a	5.62±0.03 a	0.28±0.01 a	6.59±0.02 a
fortified	5	4.87±0.08 b	0.40±0.02 b	3.61±0.02 b	4.74±0.08 b	0.43±0.01 b	4.65±0.03 b	5.07±0.11 b	0.38±0.01 b	4.70±0.02 b
	10	4.51±0.07 c	0.45±0.01 c	ND	4.44±0.07 c	0.49±0.01 c	2.34±0.02 c	4.68±0.05 c	0.44±0.01 c	2.42±0.03 c
	15	3.49±0.04 d	0.63±0.01 d	ND	3.69±0.07 d	0.59±0.02 d	ND	3.68±0.06 d	0.60±0.01 d	ND
Plum fortified	1	5.41±0.06 a	0.29±0.02 a	6.59±0.02 a	5.50±0.08 a	0.29±0.01 a	7.15±0.05 a	5.50±0.08 a	0.29±0.01 a	7.06±0.10 a
	5	4.65±0.09 b	0.43±0.02 b	4.56±0.03 b	4.94±0.04 b	0.40±0.02 b	6.55±0.03 b	4.94±0.04 b	0.40±0.02 b	5.57±0.01 b
	10	4.36±0.05 c	0.49±0.01 c	2.45±0.05 c	4.57±0.05 c	0.46±0.01 c	3.35±0.05 c	4.57±0.05 c	0.46±0.01 c	3.48±0.03 c
	15	3.33±0.08 d	0.67±0.02 d	ND	3.53±0.08 d	0.62±0.02 d	ND	3.53±0.08 d	0.62±0.02 d	ND

[1)]Data were expressed as mean±standard deviation (n=3)

[2)]When mean values were significantly different ($P < 0.05$), different letters were used (a, b, c, d)

[3)]ND= Not determined

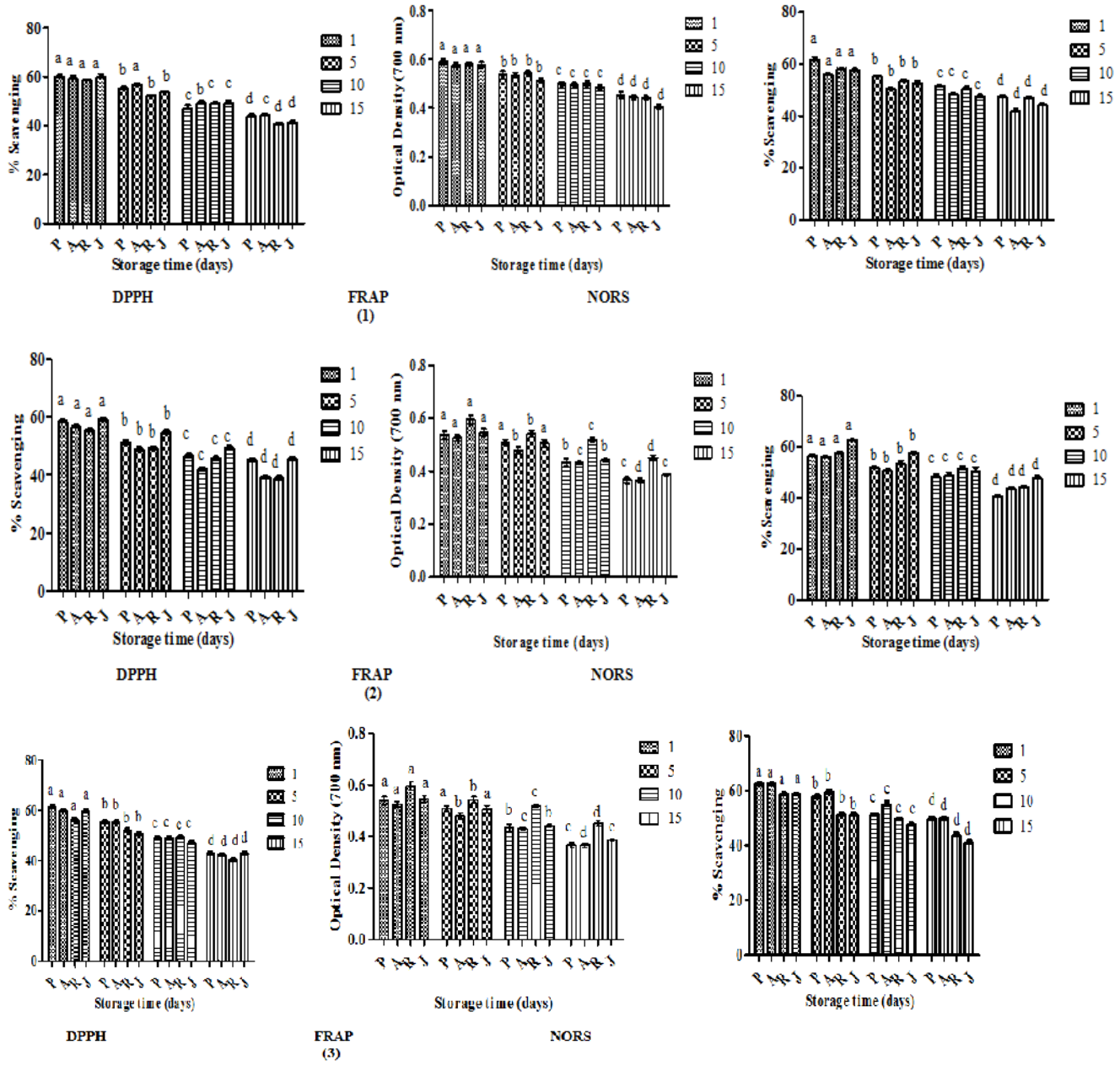

Fig. 1. Antioxidant (DPPH, FRAP, NORS) analysis of fruits fortified probiotic milk developed using 1) free *L. rhamnosus* culture, 2) alginate encapsulated probiotic culture, 3) and carrageenan encapsulated culture on 1, 5, 10 and 15 days of storage at 4ºC. P-Plum, A-Apricot, R-Raspberries and J-Jamun. When mean values were significantly different ($P < 0.05$), different letters were used (a, b, c, d).

The antioxidant activity of the fruits supplemented probiotic milk samples decreased continuously over a storage period from 1-15 days, which may be attributed to denaturation during storage time and temperature. However, all milk samples retained useful amounts of antioxidants up to 15 days of storage.

In DPPH radical scavenging assay the percentage scavenging decreased from 61.09±1.17 to 38.77±1.42 during storage. In FRAP assay the optical density decreased from 0.598±0.01 to 0.366±0.02 during storage up to 15 days. In NORS assay the percentage scavenging decreased from 62.66±0.56 to 40.61±0.90 during storage (Fig. 1).

Scibisz and Mitek (2009) reported that decrease in the anthocyanin content and antioxidant capacity of blueberry jams during storage is associated with temperature and time of storage. The antioxidant activity of the fruits and fruit jellies was related to the presence of phenolic compounds (Davalos *et al.,* 2005; Ruberto *et al.,* 2007). There is also a significant decrease of antioxidants in case of juices during storage (Wrolstad *et al.,* 2005; Ngo *et al.,* 2007). Again the reason for decrease in antioxidant activity of the fruits supplemented probiotic milk samples was attributed to denaturation during storage.

In conclusion probiotic and fruit fortified probiotic milk samples were prepared using free, alginate and carrageenan encapsulated probiotic *L. rhamnosus* culture. A continuous reduction in the viable probiotic cell count was reported during storage. However, the alginate encapsulated probiotic culture was more stable as compared with carrageenan and free probiotic culture. The antioxidant activity of the fruit fortified probiotic milk samples decreased during storage but they retained useful amount of antioxidants during storage. In future, the *in vivo* animal experiments or cell line studies and sensory studies will be required to prove the potentiality and acceptability of the products. The wild fruits used in this study can be used for the development of fruit fortified probiotic products for daily consumption.

REFERENCES

Amiri ZR, Khandelwal P, Aruna BR. (2010). Development of acidophilus milk via selected probiotics and prebiotics using artificial neural network. *Adv. Biosci. Biotechnol.* **1**: 224-231.

AOAC. (1990). Official Method of Analysis of AOAC Intl. 15th ed. Method 947.05. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Charteris WP, Kelly PM, Morelli L, Collins JK. (1998). Antibiotic susceptibility of potentially probiotic *Lactobacillus* species. *J. Food Protect.***61**: 1636-1643.

Davalos A, Bartolome B, Gomez-Cordoves C. (2005). Antioxidant properties of commercial grape juices and vinegars. *Food Chem.***93**: 325-330.

Food and Agriculture Organization/World Health Organization. (2001). Evaluation of health and nutritional properties of probiotics in food including powdered milk and live lactic acid bacteria. Available from: http://www.fao.org/ag/AGN/Probio/probio.htm.

Garrat DC. (1964). The Quantitative Analysis of Drugs. 3rd ed. Chapman and Hall, Japan. pp. 456-458.

Ghadge PN, Prasad K, Kadam PS. (2008). Effect of fortification on the physico-chemical and sensory properties of buffalo milk yoghurt. *Electron. J. Environ. Agr. Food Chem.* **7**: 2890–2899.

Heidebach T, Leeb E, Forst P, Kulozik U. (2010). Influence of casein based microencapsulation on freeze drying and storage of probiotic cells. *J. Food Eng.***98**: 309-316.

Junaid M, Javed I, Abdullah M, Gulzar M, Younas U, Nasir J, Ahmad N. (2013). Development and quality assessment of flavored probiotic acidophilus milk. *J. Anim. Plant Sci.* **23**: 1342-1346.

Kailasapathy K, Harmstorf I, Phillips M. (2008). Survival of *Lactobacillus acidophilus* and *Bifidobacterium animalis* ssp. *lactis* in stirred fruit yogurts. *Food Sci. Technol.* **41**: 1317-1322.

Krasaekoopt W, Bhandari B, Deeth H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *Int. Dairy J.***13**: 3-13.

Krasaekoopt W, Bhandari B, Deeth H. (2006). Survival of probiotics encapsulated in chitosan-coated alginate beads in yoghurt from UHT- and conventionally treated milk during storage. *Food Sci. Technol.***39**: 177–183.

Kumar A, Kumar D. (2014). Isolation and characterization of bacteria from dairy samples of Solan in Himachal Pradesh for identification of *Lactobacillus* spp. *Int. J. Pharm. Sci. Rev. Res.* **25**: 110-114.

Kumar A, Kumar D. (2015). Characterization of *Lactobacillus* isolated from dairy samples for probiotic properties. *Anaerobe.* **33**: 117–123.

Kumar A, Kumar D. (2016). Development of antioxidant rich fruit supplemented probiotic yogurts using free and microencapsulated *Lactobacillus rhamnosus* culture. *J. Food Sci. Technol.* **53**: 667–675.

Kunwar A, Priyadarsini KI. (2011). Free radicals, oxidative stress and importance of antioxidants in human health. *J. Med. Allied Sci.***1**: 53-60.

Lilly DM, Stillwell RH. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science.***147**: 747-748.

Lyras L, Cairns NJ, Jenner A, Jenner P, Halliwell B. (1997). An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. *J. Neurochem.***68**: 2061-2069.

Mohan G, Guhankumar P, Kiruththica V, Santhiya N, Anita S. (2013). Probiotication of fruit juices by *Lactobacillus acidophilus. Int. J. Adv. Biotechnol. Res.***4**: 72-77.

Mousavi ZE, Mousavi SM, Razavi SH, Emam-Djomeh Z, Kiani H. (2011). Fermentation of pomegranate juice by probiotic lactic acid bacteria. *World J. Microbiol. Biotechnol.* **27**: 123-128.

Naznin A, Hasan N. (2009). *In vitro* antioxidant activity of methanolic leaves and flowers extracts of *Lippia alba*. *Res. J. Med. Med. Sci.* **4**: 107-110.

Ngo T, Wrolstad RE, Zhao Y. (2007). Color quality of Oregon strawberries-impact of genotype, composition, and processing. *J. Food Sci.* **72**: 25-32.

Oyaizu M. (1986). Studies on products of browning reaction-antioxidative activities of products of browning reaction prepared from glucosamine. *Jpn. J. Nutr.* **44**: 307-315.

Ruberto G, Renda A, Daquino C, Amico V, Spatafora C, Tringali C, Tommasi ND. (2007). Polyphenol constituents and antioxidant activity of grape pomace extracts from five Sicilian red grape cultivars. *Food Chem.* **100**: 203-210.

Saccaro DM, Hirota CY, Tamime AY, de-Oliveira MN. (2011). Evaluation of different selective media for enumeration of probiotic microorganisms in combination with yogurt starter cultures in fermented milk. *Afr. J. Microbiol. Res.***5**: 3901-3906.

Sayre LM, Smith MA, Perry G. (2001). Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Curr. Med. Chem.***8**: 721-738.

Scibisz I, Mitek M. (2009). Effect of processing and storage conditions on phenolic compounds and antioxidant capacity of highbush blueberry jams. *Polish J. Food Nutr. Sci.***59**: 45-52.

Vodnar DC, Socaciu C, Rotar AM, Stanila A. (2010). Morphology, FTIR fingerprint and survivability of encapsulated lactic bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) in simulated gastric juice and intestinal juice. *Int. J. Food Sci. Technol.***45**: 2345-2351.

Wrolstad RE, Durst RW, Lee J. (2005). Tracking color and pigment changes in anthocyanin products. *Trends Food Sci. Technol.*, **16**: 423-428.