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ORIGINAL ARTICLE

Effect of carbon dioxide on sensory attributes, physico-chemical parameters and viability of Probiotic *L. helveticus* MTCC 5463 in fermented milk

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Abstract A carbonated probiotic fermented milk using India's first fully sequenced potential probiotic strain *Lactobacillus helveticus* MTCC 5463 in a combination with *Streptococcus thermophilus* MTCC 5460 was prepared and standardized with respect to carbon dioxide pressure, sugar and salt concentrations based on sensory, physico-chemical and microbial parameters. Final optimized product was prepared with 15 % sugar, 0.8 % salt concentration and carbonated at 15 kgcm^{-2} pressure which was subsequently subjected to shelf life evaluation for 28 days at 5±1 °C. Even though a significant decrease was observed for overall acceptability, the product was acceptable on 28th day of storage. The titratable acidity, pH and tyrosine value has increased gradually while free fatty acids remained unchanged. The viable lactobaciili and streptococci count decreased from 7.84 to 7.54 and 8.94 to 8.87 log cfumL^{-1} during 28 days of storage, respectively.

Keywords *L. helveticus* · Probiotic · Carbonation · Fermented milk · Streptococci

Introduction

Fermented foods are of great significance since they provide and preserve vast quantities of nutritious constituents in a wide diversity of aroma, flavor and texture, which enrich the human diet. Over 3,500 traditional fermented foods exist worldwide that have been considered as an important component of diet from ages (Prajapati and Nair 2003). Among various raw materials used for fermentation, milk has been widely employed for the manufacturing of fermented products because of its well established nutritional value. As per one estimate, 9 % of the total milk produced in India is converted into fermented milk products (Prajapati 2011). Carbonated fermented milk products such as Kefir and Koumiss are well known in the regions between Eastern Europe and Mongolia due to their therapeutic benefits and sparkling alcoholic taste (Prajapati and Nair 2003). These products are not so popular in the other part of the world due to the lack of awareness among people, associated alcoholic taste and flavor of the products; and uncontrolled fermentation.

The society is becoming more conscious about health and nutrition and has experienced hazardous effects of soft drinks; hence, a special niche for self carbonated nutritious drink is being created (Shah et al. 2009). The consumer demands for nutritive, hygienic and healthy products have made the processing and packaging technology much more innovative and effective. The trend has shifted towards the food grade gases such as carbon dioxide and nitrogen in order to achieve preservatives free and fresh food products. Carbon dioxide is a naturally present component of freshly drawn milk in the dissolved form (40 to 1,100 ppm) which immediately dissipates upon heat treatment. Carbonation is one of the popular methods of preserving and increasing the shelf life of various foods including milk (Khurana and Kanawjia 2007). Addition of carbon dioxide is practiced since long for the preservation of raw milk and milk products such as dry milk powders, cheeses, yoghurt and fermented beverages (Espie and Madden 1997; Eliot et al. 1998; Noriega et al. 2003; Oyugi and Buys 2007). Carbonation of fermented milks enhances the acceptability of fermented dairy products by providing thirst

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quenching and refreshing nature which can subsequently place them as a viable, novel and healthy alternate over the carbonated soft drinks.

The market of probiotic is growing rapidly due to awareness in the society about their beneficial health effects (Vinderola et al. 2000). Among probiotic cultures, lactobacilli and bifidobacteria are extensively used due to their capability of survival into gastrointestinal (GI) tract. Milk is an excellent matrix to carry probiotic organisms. Fermentation of milk further enhances its acceptability. Several researchers had worked on diverse fruit flavored as well as plain carbonated yoghurt (Choi and Kosikowski 1985; Yau et al. 1989; Lederel et al. 1991). Some of the workers have formulated the product using the probiotic strains either of *Bifidobacterium* spp. and/or *Lactobacillus* spp. in combination with streptococci to study their symbiosis, effect on harmful bacteria and on sensory attributes (Karagul-Yuceer et al. 1999; Vinderola et al. 2000; Noriega et al. 2003).

A potential probiotic strain *L. helveticus* MTCC 5463, India's first fully sequenced isolate (Prajapati et al. 2011), has been proven for strong antimicrobial effect against various Gram positive and Gram negative bacteria and its cytotoxicity against HeLa and HEp-2 tumor cells (Khedekar et al. 1990a, b). It has been studied for hypocholesterolemic effect in humans (Ashar and Prajapati 2001); immunostimulating activity in chicks (Patidar and Prajapati 1999) and adhesion capabilities in the CaCo-2 cell lines (Kodaikkal et al. 2012). Another strain *S. thermophilus* MTCC 5460 is a dahi isolate, extensively studied for manufacturing of dahi, lassi, and yoghurt, either alone or in combination with lactobacilli (Prajapati et al. 1995; Patidar and Prajapati 1998). Present investigation was undertaken to formulate a novel probiotic carbonated fermented milk employing these two indigenous cultures.

Materials and methods

Bacterial strains and culture media

Streptococcus thermophilus MTCC 5460 and *Lactobacillus helveticus* MTCC 5463 were obtained from the culture collection of Dairy Microbiology Department, Anand Agricultural University, Anand, India. They were maintained and propogated in sterilized reconstituted skim milk (10 % Total Solids) followed by incubation at 37 °C for 8 h and stored at 5±1 °C during the entire course of the study. Prior to use, three successive transfers of cultures were given in skim milk to activate them. All bacteriological media were purchased from Sigma (Germany) or Hi-Media (Mumbai, India) while analytical grade chemicals were obtained from Merck (Germany) or SDFCL (Mumbai, India).

Carbonated probiotic fermented milk

The standard protocol as suggested by Patidar and Prajapati (1998) with slight modification was followed for the preparation of fermented milk. The modifications included dilution of lassi with water (50 %v/v of milk), carbonation and packing in crown cropped bottles. For preparation of product, double tonned milk (1.5 % fat, 9.0 % SNF), good quality sugar, salt and stabilizers were purchased from local market (Anand, India). The carbonation machine, crew cropped bottles and capping machine were procured from local supplier Rana Traders, Anand, India.

Optimization of carbonation, sugar and salt level

Carbonation

Fermented milk prepared by incorporation of 10 % sugar was used for optimization of carbonation pressure. The product was filled in 200 mL glass bottles and carbonated at 10, 15 and 20 $kgcm^{-2}$ pressure. The best carbonation treatment was decided based on sensory scores, physico-chemical parameters and probiotic lactobacilli count.

Sugar

Fermented milk was prepared by addition of 10, 12 and 15 % sugar (w/v) of the milk taken and carbonated at 15 $kgcm^{-2}$. The best sugar level was decided based on sensory scores, physico-chemical parameters and probiotic lactobacilli count.

Salt

A plain fermented milk was prepared as reported by Patidar and Prajapati (1998) and after stirring NaCl was incorporated at 0.5, 0.8 and 1.0 % (w/v) in three different lots. The products were filled in 200 mL bottles and then carbonated at 15 $kgcm^{-2}$. The optimum salt level was judged based on sensory scores, physic-chemical parameters and probiotic lactobacilli count.

Sugar and salt

To study the effect of sugar and salt added together, three sets of product viz., 1) product prepared with 15 % sugar only, 2) product prepared with 0.8 % salt only and 3) product prepared with 15 % sugar and 0.8 % salt were carbonated at $kgcm^{-2}$ pressure. These products were evaluated based on sensory scores.

Shelf life study

Final product was formulated based on results of optimization for CO_2, salt and sugar incorporation level. The product was stored at 5±1 °C and analyzed periodically at 7, 14, 21 and 28 days for sensory scores, physico-chemical parameters and probiotic lactobacilli count to determine its shelf life in three independent trials.

Analysis of product

Sensory evaluation

A panel of nine discriminative and experienced experts from the Faculty of Dairy Science, AAU, was formulated. All the samples were evaluated for sensory attributes such as color and appearance, body and texture, flavor, and overall acceptability on a nine-point hedonic scale (9 for liking extremely and 1 for disliking extremely). The products were marked randomly and served in 200 mL bottles at 5±1 °C in a specially designed sensory evaluation booths.

Microbial analysis

Serial dilutions of the product were prepared using phosphate buffer and appropriate dilutions were pour-plated in duplicate on a selective media, de Mann-Rogosa-Sharpe (MRS) agar for lactobacilli count and M17 agar for streptococci count. All plates were incubated at 37 °C up to 72 h. After incubation periods, plates were observed for colony characteristics and enumerated. Yeast and mold count (YMC) and coliform count (CC) were also carried out to judge the hygienic conditions during the product manufacturing process. Potato dextrose agar (PDA) adjusted to pH≤3.5 with 10 % sterile tartaric acid was used for plating and the plates were incubated at 25 °C for 3–5 days. Coliform count was done on Violet red bile salt agar (VRBA) by incubating the plates at 37 °C for 24 h.

Chemical analysis

The titratable acidity of carbonated fermented milk was measured as percent lactic acid from the product during the entire study as per the method of IS: 1469, part I (1960), using 10 mL of sample. pH was determined using Electronic pH meter (Model CYBERSCAN 2100, Singapore). Percent of settling was calculated by measuring the separated water from 200 mL bottle of the product during different intervals of storage. The proteolytic and lipolytic activity of LAB were determined in terms of tyrosine value (modified Hull method 1947) and free fatty acid value (Deeth and Fitz-Gerald 1976), respectively.

Composition of product

Total solids (T.S.) were determined gravimetrically according to the procedure described in IS: 1479 (II) (1961). Fat content of sample was determined as per the procedure given in Indian Standards (1981a) by Gerber method, whereas, protein content of product sample was estimated by Kjeldahl method as described in IS: 9617 (1980). Total carbohydrates were determined by difference (IS (SP: 18 (part XI), 1981b) while calcium content was measured according to method given in IS (SP: 18 (Part XI) 1981b).

Statistical analysis

All experiments were carried out in triplicate (n=3). Data were analyzed for variance by Completely Randomized Design (CRD) as per the methods described by Steel and Torrie (1980). The significance was tested at five percent level using 'F' test and CD percent was calculated. The values for microbial counts were log transformed before analysis.

Results and discussion

The product was prepared by fermentation of milk with *S. thermophilus* MTCC 5460 (a dairy starter culture) and *L. helveticus* MTCC 5463 (a potential probiotic culture). Both the cultures are known to grow in symbiotic association in milk (Prajapati et al. 1995; Patidar and Prajapati 1998). While development of a carbonated fermented milk in a beverage form, it was essential to optimize the level of carbonation as well as sugar and salt concentration to make it sensorily appealing.

Effect of carbonation

Effect of different levels of CO_2 on sensory attributes of a probiotic carbonated fermented milk is shown in Table 1. The flavor, body & texture, color & appearance and overall acceptability scores were higher for 15 $kgcm^{-2}$ (P3) as compared to 10 and 20 $kgcm^{-2}$ pressure. However, out of all the parameters, differences in color and appearance only were significant (P<0.05). Significantly lower score for P3 may be due to uneven distribution of gel particles which resulted due to high carbonation pressure leading to poor appearance. An average overall acceptability score was highest with P2 (7.69) followed by P3 (7.44) and P2 (7.66). Extent of carbonation in the product and the feel of carbonation by judges did not coinside. Product made with 20 $kgcm^{-2}$ pressure showed the lowest carbonation feel (7.18) while the product with 15 $kgcm^{-2}$ pressure showed highest (7.58) feeling of carbonation. This indicates that the

Table 1 Sensory profile of carbonated fermented milk prepared with different levels of carbon dioxide pressure, sugar and salt

Parameter	Sensory attributes (Score out of 9)				
	Flavour	Colour and Appearance	Body and Texture	Overall acceptability	Carbonation
P1 (10 $kgcm^{-2}$)	7.5±0.62	7.7±0.77[a,b]	7.2±0.73	7.4±0.45	7.3±0.77
P2 (15 $kgcm^{-2}$)	7.8±0.69	7.9±0.58[a]	7.4±0.51	7.7±0.66	7.6±0.52
P3 (20 $kgcm^{-2}$)	7.3±0.69	7.2±0.58[b]	7.3±0.51	7.7±0.66	7.2±0.52
S1 (10 %)	6.9±0.94[a]	7.1±0.87	7.0±0.86	7.1±0.82[a]	–
S2 (12 %)	7.5±0.92[b]	7.5±0.88	7.3±0.84	7.5±0.86[a]	–
S3 (15 %)	8.1±0.85[c]	7.8±0.87	7.8±0.83	8.2±0.85[b]	–
N1 (0.5 %)	7.6±0.75	7.7±0.63	7.6±0.72	7.6±0.72	–
N2 (0.8 %)	7.9±0.71	7.9±0.67	7.6±0.75	7.9±0.67	–
N3 (1.0 %)	7.3±0.66	7.6±0.61	7.3±0.76	7.4±0.59	–

±; Standard deviation,

n=3 (each replicate is average score from 9 panelists),

Values with different super script in each column and each set of treatments, viz., carbonation pressure (P), sugar level (S) or salt level (N) differ significantly at 5 % level

absorption of carbon dioxide in the product may not be directly proportional to the pressure applied. Generally in food products, the absorption of CO_2 depends on the nutrients and different physico-chemical forms of the ingredients. In the previous studies, CO_2 pressure levels used varied between 0.5 $kgcm^{-2}$ and 72.48 $kgcm^{-2}$ in carbonated yoghurt and whey beverages (Choi and Kosikowski 1985; Sheikh et al. 2001). Gueimonde and de los Reyes-Gavilan (2004) showed non-significant effect of the carbon dioxide on sensory properties as well as on starter cultures used in the preparation of carbonated fermented milk.

It is expected that forcing CO_2 into the product will affect its settling property significantly. Hence, the rate of settling of the product in terms of the quantity of separated water during storage of the product in undisturbed condition was measured. With the increase in carbonation pressure, the percentage of settling of the product also increased after 24 h of holding at refrigeration temperature (Table 2). High CO_2 pressure enforces rapid serum separation in the product by making free bounded water through the breakage of chemical bonding between water and stabilizer. According to Yau et al. (1989), carbonation cause the rapid separation of curd and serum layer which occurred in about equal parts when stored at 0 to 1 °C for 2 days and therefore the shaking of bottles is required before consumption. The initial titratable acidity of product before carbonation was 0.54 % L.A, with pH of 4.65. As it is indicated in Table 2, the increase in titratable acidity was minute with simultaneous increase in the level of carbon dioxide. Development of carbonic acid leads to further fall in pH. Both the groups of lactic acid bacteria were not significantly affected by any of the CO_2 level. The viable lactobacilli count ranged from 7.66 to 7.78 log $cfumL^{-1}$, while streptococci count ranged from 8.68 to 8.86 log $cfumL^{-1}$ (Fig. 1a). Karagul-Yuceer et al. (2001) had prepared the carbonated yoghurt with two different yoghurt cultures (YC 470 and YC 180). They found that the survival of both the cultures was 8.65 and 9.18 log $cfug^{-1}$, respectively, at first day of storage.

Effect of sugar and salt addition

Generally, the sweetening agent acts as an enhancer of acceptability in all food products. For all the sensory attributes the score was higher for the product with 15 % sugar (S3) followed by 12 % (S2) and 10 % (S1) as shown in Table 1. The product with 15 % of sugar was liked better by

Table 2 Effect of carbonation, sugar and salt addition on physico-chemical parameters of carbonated fermented milk

Parameter	% lactic acid	pH	% Settling
P1 (10 $kgcm^{-2}$)	0.6±0.01	4.6±0.02	70±2.01
P2 (15 $kgcm^{-2}$)	0.6±0.01	4.6±0.01	73±1.53
P3 (20 $kgcm^{-2}$)	0.6±0.02	4.6±0.01	75±0.55
S1 (10 %)	0.5±0.02	4.6±0.02	75±1.25
S2 (12 %)	0.6±0.02	4.6±0.02	73±2.35
S3 (15 %)	0.6±0.03	4.5±0.03	70±1.23
N1 (0.5 %)	0.6±0.01	4.6±0.01	71±2.28
N2 (0.8 %)	0.6±0.01	4.6±0.01	73±1.53
N3 (1.0 %)	0.6±0.03	4.6±0.02	75±0.80

±; Standard deviation,

n=3,

None of the values in any of the parameters in different set of treatments, viz., carbonation pressure (P), sugar level (S) or salt level (N) differ significantly at 5 % level the judges as it gave almost perfect combination of sweetener and CO_2 pressure level. The average overall acceptability scores were 8.22, 7.52, and 7.11 for S3, S2, and S1, respectively.

Karagul-Yuceer et al. (1999) found that the product with sucrose as sweetener was liked by judges at 60 % for overall acceptability followed by High Fructose Corn Syrup (HFCS) (58 %) and aspartame (40 %). It is known that the incorporation of sugar controls the settling of total solids in the product due to its well known water binding capacity. Thus, product with higher sugar content has shown less degree of whey separation compared to others (Table 2). In contrast, highest titratable acidity (0.61 %L.A.) was observed with higher percent of sugar. The increase in acidity observed could be because of more availability of carbohydrates to ferment it into acids. There was no change in the count of LAB observed due to different sugar levels. Choi and Kosikowski (1985) reported that addition of sugar did not stimulate or inhibit the viable count of streptococci and lactobacilli in carbonated yoghurt.

In general, salt can improve the taste as well as flavor of the product. None of the sensory parameters significantly differ ($P<0.05$) with respect to incorporation of salt at different levels (Table 1). Even though, non-significant, all the attributes were marginally better with 0.8 % salt level. Addition of salt was at lower concentration and hence it did not influence either chemical or microbial parameters. The average viable count of streptococci and lactobacilli was ranging from 8.65 to 8.88 cfumL^{-1} and 7.75 to 7.87 cfumL^{-1} (Fig. 1a), respectively.

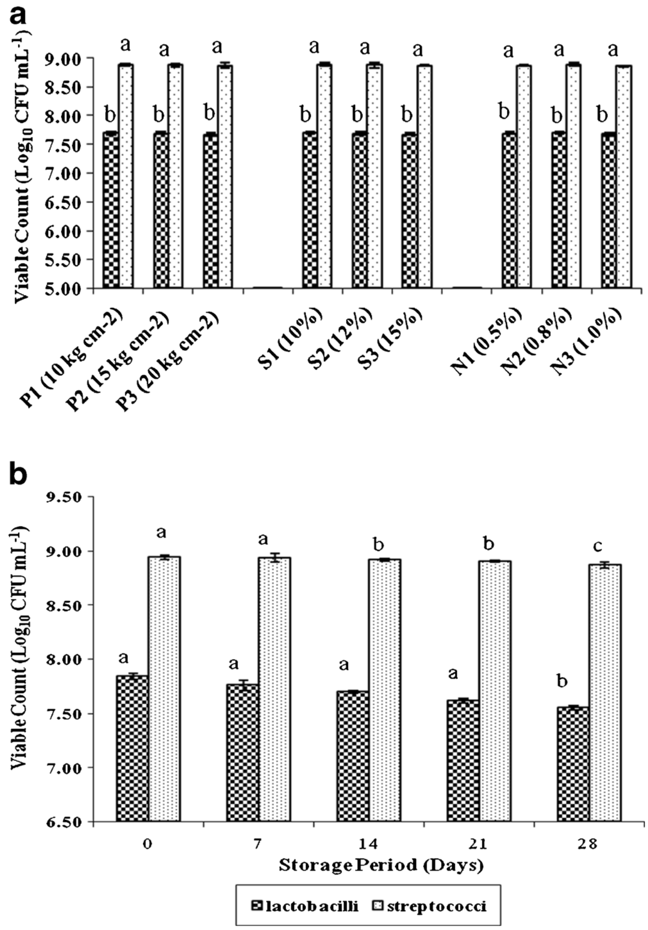

Fig. 1 Effect of different carbon dioxide pressure, sugar and salt concentrations (**a**) and storage at 5±1 °C (**b**) on viable count of lactobacilli and streptococci in carbonated fermented milk (n=3). ±; Standard deviation, n=3. Values with different super script differ significantly at 5 % level. All the values of streptococci or lactobacilli count in Figure (A) did not differ significantly at 5 % level in any of the treatments, viz., carbon dioxide pressure (P), sugar (S) or salt (N)

Combination of sugar and salt

Considering the comments of sensory evaluation panel, it was decided to formulate a product with combination of 15 % sugar and 0.8 % salt for getting still better acceptability. The comparison of sensory score of the product prepared with either sugar or salt and a combination thereof are given in Table 3. Overall acceptability score of salt and sugar added product was significantly ($P<0.05$) superior (8.08) than only salt added (6.88) or only sugar added (7.52) product. Irrespective of the scores, most of the judges put a remark that a product with sugar and salt gives a better sparkling taste and improved aroma. Thus, looking to all results and comments obtained, the final product was formulated with 15 kgcm^{-2} carbonation pressure level, 15 % sugar and 0.8 % salt.

Composition of carbonated fermented milk

The proximate composition of the final standardized product was determined. The average moisture, fat, protein, carbohydrates and calcium content of the product was 87.5, 0.71, 3.2, 8.02 and 0.048 %, respectively.

Table 3 Sensory profile of carbonated fermented milk prepared with salt, sugar or combination thereof

Parameter	Sugar (S)	Salt (N)	Salt (N) and Sugar (S)
	Score out of 9		
Flavor	7.6±0.72	7.3±0.54	7.6±0.75
Color and Appearance	7.7±0.77	7.6±0.72	7.9±0.58
Body and Texture	7.6±0.75	7.3±0.66	7.9±0.55
Overall acceptability	7.5±0.92^{a}	6.9±0.94^{b}	8.1±0.76^{c}

±; Standard deviation,

n=3 (each replicate is average score from 9 panelists), values with different super script in each row differ significantly at 5 % level

Shelf life study

Sensory evaluation

Usually, the shelf life of non-sterile dairy product is limited to 15–18 days at refrigeration temperature (Salvador and Fiszman 2004). Hence, in order to examine positive effects of carbonation in terms of improved shelf life and overall acceptability, the final product was stored at commercial refrigeration temperature (5±1 °C) for 28 days and assessed for microbial, physico-chemical and sensory parameters. The initial average score remained same for all parameters. Body and texture and overall acceptability showed significant ($P<0.05$) decline throughout the storage period whereas the sharp decrease in flavor score was reported between 14 and 28 days of storage. On 28th day of storage, score for flavor, color and appearance. body and texture and overall acceptability was 6.95, 7.54, 7.51, and 7.52, respectively (Table 4).

The probable causes for significant reduction in acceptability scores would be appearance of flaxes in the product, development of non-uniform curd particles, hardening of fat globules, sticking of the product at the walls of glass container, increase in acidity, continuous proteolysis, lack of freshness, after 14 days of storage. Nevertheless, product was still acceptable by judges on the 28th day of storage. Owing to higher dissolving rate of CO_2 at low temperature, it could have boosted higher acceptability (Singh et al. 2012). On the contrary, Karagul-Yuceer et al. (1999) suggested that during storage of carbonated yoghurt, carbonation did not affect the flavor of the product. Their product scored 7.50, 6.10 for flavor and body and texture, respectively, which is lower in comparison to our score (8.22). They also stated that the carbonated yoghurt was mostly preferred by judges (54 % out of 60 %) as compared to noncarbonated yoghurt. Choi and Kosikowski (1985) suggested that the carbonated yoghurt was acceptable for 4 months compared to non carbonated yoghurt which remained good for 30 days at 4.4 °C and the carbonated and non carbonated yoghurt stored at 10 °C were acceptable for 45 days and 25 days, respectively.

Chemical and microbiological analysis

The titratable acidity of the product significantly ($P<0.05$) increased from 0.56 % L.A. to 0.91 % L.A at the end of 28 days storage with the simultaneous decrease in the pH from 4.66 to 4.33. A quick drop in pH was observed after 14 days of storage (Table 4). The probable reason for this drop may be continuous metabolic activity of LAB at slower rate under refrigeration temperature. In addition to this, development of carbonic acid in aqueous phase of the product decreases pH (Singh et al. 2012). Almost, similar results were observed by Choi and Kosikowski (1985). They reported increase in titratable acidity of the order of 0.20 % in carbonated and 0.50 % in noncarbonated yoghurt stored at 4.4 °C for 28 days while the pH was 3.72 in the carbonated yoghurt at the end of 35 days.

The tyrosine value of the product significantly ($P<0.05$) increased from 2.08 to 2.89 mg/100 g during storage of 28 days. This could be due to the onset of proteolysis by starters themselves or mediated by enzymatic hydrolysis of certain metabolites. On the other hand, free fatty acid value (0.6 μeq/g) remained unchanged during entire storage period which indicates no lipolytic changes in the product. Choi and Kosikowski (1985) reported that free fatty acids in carbonated yoghurt beverages did not change during first 20 days of storage at 4.4 °C. No changes in free fatty acids value were apparent because the product had very less fat content and was free from lipolytic organisms like yeast and mold and also not exposed to light.

Survival of LAB, development of off flavor and yeast and mold growth (YM) are the important parameters to estimate keeping quality of fermented milk products (Robinson 2002; Patel and Prajapati 2010). Four different microbiological counts viz. streptococci, lactobacilli, coliform and yeast and mold were carried out to assure the microbiological quality of

Table 4 Effect of refrigerated storage (5±1 °C) on sensory quality and physicochemical characteristics of carbonated fermented milk

Parameter	Period (Days)				
	0	7	14	21	28
	Sensory quality (*n*=3 replicates and average score from 9 panelists)				
Flavor	$8.6±0.48^a$	$8.4±0.51^b$	$8.2±0.56^c$	$7.5±0.46^d$	$6.9±0.68^e$
Color & Appearance	$8.6±0.53^a$	$8.4±0.58^b$	$8.3±0.46^{b,c}$	$8.2±0.52^c$	$7.5±0.53^d$
Body & Texture	$8.6±0.66^a$	$8.4±0.52^b$	$8.3±0.46^{b,c}$	$8.2±0.66^c$	$7.5±0.60^d$
Overall acceptability	$8.6±0.52^a$	$8.4±0.61^b$	$8.3±0.46^{b,c}$	$8.2±0.59^c$	$7.5±0.55^d$
	Physicochemical (*n*=3)				
Acidity (% lactic acid)	$0.6±0.01^a$	$0.6±0.03^a$	$0.7±0.02^b$	$0.8±0.03^c$	$0.9±0.02^d$
pH	$4.7±0.01^a$	$4.6±0.02^b$	$4.6±0.02^b$	$4.4±0.01^c$	$4.3±0.03^d$
Tyrosine mg 100 mL^{-1}	ND	$2.1±0.06^a$	$2.3±0.03^b$	$2.6±0.03^c$	$2.9±0.07^d$

±; Standard deviation,

Values with different super script in each row differ significantly at 5 % level

ND not determined

the product. The initial viable count of streptococci (8.94 log cfumL^{-1}) was reduced to 8.87 log cfumL^{-1} at the end of 28 days which was non-significant between 0 to 7 and 14 to 21 days but significant ($P<0.05$) on 7th and 28th days, respectively (Fig. 1b). Diminished Streptococcal count was attributed to lack of nutrients, age of cells, and production of certain toxic metabolic compounds. The results are in harmony with Karagul-Yuceer et al. (2001), who showed the gradual decrease in viable count of streptococci from 8.65 to 8.58 log cfug^{-1} during the storage period of 21 days. The lactobacilli count non-significantly decreased from 7.84 to 7.55 for the first 21 days of storage (Fig. 1b). Lactobacilli show comparatively high resistance, tolerance and growth to/in carbon dioxide, even up to 100 % as mentioned by Hendricks and Hotchkiss (1997). Shaw and Nicol (1969) illustrated least inhibitory effect of CO_2 with Gram-positive psychrotrophs, particularly *Lactobacillus* spp. Similarly, Karagul-Yuceer et al. (2001) premised that carbon dioxide facilitate the growth of starter cultures thereby supporting their production time.

Yeast, mold and coliform counts (cfumL^{-1}) were carried out from fresh as well as stored products. It was found that during entire course of study, both contaminants were absent in 1 mL of product. Absence of yeast, mold and coliform indicates excellent hygienic environmental conditions during the product manufacturing till the end of storage period.

Conclusion

Rising public demand for healthy and innovative products with added nutritive advantages has created new horizon for food and dairy industry. Special niche for self carbonated nutritious drink is emerging in the society. Carbon dioxide is being used as an effective preservative in food industry that acts as an enhancer of the sensory attributes of the product. Since, naturally carbonated fermented milk has a problem of uncontrolled fermentation and a typical fermented flavor, external incorporation of CO_2 serve as an efficient and convenient alternate to get carbonated product. A carbonated probiotic fermented milk could be successfully standardized with shelf life of 28 days at 5±1 °C with viable probiotic lactobacilli count of 7.55 log cfumL^{-1} at the end of shelf life.

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