Carbonated fermented dairy drink – effect on quality and shelf life

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Abstract Processing conditions were standardized for a carbonated sweetened fermented dairy beverage. The optimum level of carbonation for the beverage filled in 200 ml glass bottles was found to be at 50 psi pressure for 30 seconds. The beverage samples were stored under refrigerated conditions (7 °C) and evaluated at weekly intervals for their sensory, chemical and microbial quality. The uncarbonated control samples were found to keep well till 5 weeks of storage while the carbonated beverage was acceptable up to 12 weeks of storage. Carbonation did not significantly alter the pH of the beverage, while a marginal increase in titratable acidity was recorded for the carbonated samples. Carbonation was found to arrest the development of lipolysis and proteolysis in the beverage during storage. Microbiological investigations established the inhibition of yeast and mold growth due to dissolved CO₂.

Keywords Fermented milk · Carbonation · Quality · Shelf life

Introduction

Cultured butter milk, a byproduct in the traditional manufacture of butter (*makhan*), today is commonly produced from skim or whole milk, fermented using starter culture and suitably diluted. The fermentation process is believed to increase the shelf-life of the product, as well as add to the taste and improved digestibility of the milk solids. In India, it is estimated that around 9 % of the total milk produced is converted into fermented milk products with an annual

growth rate of more than 20 % per annum for this sector (Singh 2007). Sweetened cultured buttermilk, popularly known as *lassi*, is commonly consumed as a refreshing beverage in India. Salted and spiced cultured butter milk (colloquially known as *chaas*, *majjiga*, *sambhaaram* etc) is also very popular, especially in the southern parts of the country. Other fermented milk products, reported in literature, include yoghurt, acidophilus milk, sour cream, kefir, koumiss, labneh etc. (Goff 1995; Khurana and Kanawjia 2007). Generally, non sterile fermented dairy beverages have a shelf life of 2–3 days at room temperature (Ramana and Tiwari 1999; Behare and Prajapati 2007), while they remain acceptable for 2–3 weeks under refrigerated storage (Patidar and Prajapati 1998; Salvador and Fiszman 2004); aseptically packed UHT treated fermented milks have a shelf life of about 120 days at room temperature.

The major mechanisms of spoilage of most dairy products are microbial, since milk solids act as a good environment for their growth (Hotchkiss et al 2006). The major spoilage organisms for dairy products include aerobic psychrotrophic Gram-negative bacteria, yeasts, moulds, heterofermentative lactobacilli, and spore-forming bacteria (Ledenbach and Marshall 2009). Carbon dioxide (CO₂) is known to exert an inhibitory effect on the growth of microbes (Dixon and Kell 1989). In the beverage industry, carbonation leads to dissolved CO₂, which in addition to its antimicrobial action, gives the product a sparkling appearance, astringency and a refreshing aftertaste. The exact mechanism of the antimicrobial action of dissolved CO₂ is not properly understood and can be attributed to several factors. Dixon et al (1987) proposed that interference by CO₂ in the biochemical pathways may adversely affect microbial growth. Daniels et al (1985) postulated that the growth rate of aerobic bacteria was hindered due to the displacement of oxygen by CO₂ in the product. In aqueous media, CO₂ forms carbonic acid that liberates H⁺, resulting in a reduced pH and

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increased acidity of the beverage, creating a stress in the microbial environment and adversely influencing its physiological activity (Wolfe 1980; Karagul-Yuceer et al 2001). The role of CO₂ in inhibiting microbial activity by alteration and regulation of enzymes has also been reported (Pichard et al 1984).

CO₂ is Generally Recognized As Safe (GRAS) (FDA 2011) and the dissolved gas is a natural component of freshly drawn milk that is subsequently lost during processing and transit. Hence, it can be utilized as a simple, inexpensive method to improve the shelf life of dairy products. Carbonation of dairy beverages is reported to improve its thirst quenching and refreshing quality, positioning dairy beverages as a novel and nutritive beverage, appealing to the younger generation as a healthy alternative to carbonated soft drinks. Carbonation has already been reported for extension of shelf life of fluid milk / flavoured milk (Hotchkiss and Lee 1996; Ravindra et al 2011) and dairy products like yoghurt (Karagul-Yuceer et al. 1999), ice cream mix (Hotchkiss and Chen 1996) and cheese (Mann 1991). The present study pertains to investigations on use of carbonation as a means to extend the shelf life of a sweetened fermented dairy drink and its effect on the chemical and microbial quality of the beverage.

Materials and methods

Preparation of fermented dairy drink (FDD)

Raw cow's milk procured from the Experimental Dairy Plant of the Institute was standardized to 3.5 % fat and 8.5 % SNF. The milk was heated at 85 °C for 30 min followed by cooling to 30 °C. Dahi culture (*Lactococcus lactis* spp. *lactis*) sourced from the Dairy Bacteriology Section of the institute was added to the milk at the rate of 2 % and the mix was incubated at 30 °C for 18 h till the curd was firmly set. Sugar (15 % milk basis) along with 0.3 % guar gum (stabilizer) was dissolved in boiling water (10 % milk basis), filtered and cooled to yield the sugar syrup. The level of addition of sugar and stabilizer was based on our preliminary evaluations and are in agreement with the levels reported in literature (George et al 2010; Nair and Thompson 2008). The set curd was broken by mild agitation and carefully blended with the sugar syrup. The FDD, thus prepared, was homogenized at 150 psi in a APV Crepaco homogeniser. The fermented dairy drink was cooled to 7±1 °C and filled into sanitised glass bottles of 200 ml capacity for carbonation and storage.

Carbonation procedure

The system for injecting carbon dioxide in the beverage for the study was developed by modifying a domestic carbonator

(Mr. Butlers Instafizz, Kerala). The carbonator was fitted with a 1 kg CO₂ gas cylinder along with necessary attachments of regulators and pressure gauges. The system was leak-tested before each trial using the standard soap bubble test. The glass bottle containing FDD was placed on the system platform such that the gas injection pipe remained dipped in the sample during carbonation. The bottle was raised using the platform lever of the carbonator to snugly fit to the top seal ring. The pressure of the gas in the injection line was manually adjusted to required levels and the gas was then flushed into the beverage by pressing the injection button for the required time. At the end of carbonation, the bottle platform was lowered; the bottle collected and immediately capped with gas leak proof screw caps.

Storage studies

The carbonated FDD prepared as per the standardized method was evaluated for its shelf life under refrigerated conditions (7±1 °C) against an uncarbonated control. The samples were regularly evaluated for their quality at weekly intervals. The refrigerated control and carbonated samples (about 50 ml) were served to a sensory panel of 10 panelists from among the institute staff to evaluate its sensory attributes in terms of its colour and appearance, flavor, body and texture and overall acceptability using a 9-point hedonic scale (BIS 1971).

Physico-chemical and microbial analysis

The physico-chemical attributes of fermented dairy drink were evaluated for each sample during storage. pH (Potentiometric method, Systronics digital pH meter: MK - VI) and titratable acidity (BIS 1981) were determined for both the carbonated and uncarbonated samples. The effect of carbonation on lipolysis and proteolysis, were recorded in terms of the Free Fatty Acid (FFA) (Deeth et al 1975) and soluble nitrogen (Rowland 1938) contents of the stored samples, respectively. The microbiological quality of the samples was evaluated in terms of Standard Plate Count (SPC) and Yeast and Mould Count (YMC) following the procedures described in Houghtby et al 1993. SPC was determined by plating in duplicate in SPC agar incubated at 37 °C for 24–48 h. Similarly, YMC was enumerated by plating in potato dextrose agar. Duplicate plates were incubated at 30 °C for 42–72 h and colonies with visual growth were counted and expressed as cfu/ml of the product.

Statistical analysis

The experiments were conducted using a fully factorial design of 2×13×3 for 2 treatments (with and without carbonation) and 13 storage periods (0–12 weeks) in triplicate.

Statistical analysis of the effect of carbonation and storage period on the sensory, chemical and microbial profile of the stored beverage included one way ANOVA followed by Tukey's HSD test using SYSTAT 8.0. The homogeneity of variances of the means of each experiment was checked using Hartley's test, $p < 0.01$ (Granato et al 2010). Departures from assumptions of homoscedasticity were attempted to be rectified by logarithmic transformation of data, failing which (Hartley's test $p < 0.01$ even on transformed data), the data was identified as inherently heteroscedastic (Coutteau et al 1994).

Results and discussion

Selection of gas pressure – injection time for carbonation

Preliminary studies to identify the optimum combination of gas injection pressure and time for the fermented dairy drink were carried out by carbonating the beverage at different combinations of injection pressure (10–90 psi, at increments of 10 psi) and time (10–120 s, at increments of 10 s) and the sensory threshold for the degree of carbonation was evaluated subjectively. The corresponding pH of the carbonated beverage was also recorded. From this preliminary investigation, it was deduced that carbonation did not significantly alter the pH of the beverage. However, the sensory scores for flavour and overall acceptability of the carbonated product were significantly affected beyond a threshold limit for “fizziness” observed in the product. Based on these investigations, a pressure – time combination of 50 psi and 30 s (for a sample size of 200 ml) was selected for carbonating the beverage at 7 °C.

Changes in the quality of FDD during storage

The carbonated and control samples were evaluated to investigate the effect of carbonation on the sensory, chemical and microbial quality of the sample.

Changes in sensory quality

The sensory quality of the carbonated product and control under refrigerated storage was judged using a 9-point hedonic scale at weekly intervals and the results are presented in Table 1. The initial scores for flavour, body and texture and overall acceptability of the carbonated beverage were marginally lower than the control sample (7.87–7.92 for carbonated sample against 8.00–8.03 for control). This was attributed to the “fizz” in the carbonated product, which is generally not associated with fermented dairy beverages. The initial difference between the control and carbonated, in flavour, body and texture and overall acceptability, was statistically significant.

During the 4th week, the flavour score for the control diminished and it was rejected in the 5th week due to pronounced off flavour, while the carbonated sample was found retaining its sensory attributes better than the control. The carbonated samples were found to lose their freshness by the 10th week and hence were observed to obtain diminishing scores during sensory evaluation. The carbonated samples were acceptable up to 12 weeks of storage. Thus, a significant improvement in shelf life of the beverage was achieved by the simple technique of carbonation. The results of statistical analysis of the sensory scores are presented in Table 1. It was observed that while carbonation did not significantly influence the sensory scores for colour and appearance, it significantly influenced the scores for flavour, consistency and overall acceptance. Storage period influenced all the sensory parameters significantly.

Changes in chemical quality

The effect of carbonation on the chemical attributes of flavored fermented drink evaluated during storage is presented in Fig. 1. Carbonation did not significantly alter the initial pH of the beverage, probably due to the buffer capacity of milk (Hotchkiss and Lee 1996). pH values for control and carbonated beverages were 4.51 and 4.47, respectively. However, a marginal increase in the acidity of the sample was observed on carbonation. A similar influence of carbonation was reported on the pH and titratable acidity for yoghurt beverage (Choi and Kosikowski 1985). There was a marginal decrease in the pH value of the carbonated sample reaching 3.83 after 12 weeks of storage. On storage, the control sample progressively became acidic resulting in an acidity value of 1.05 % LA (lactic acid) during the 5th week. The carbonated sample, which had an initial acidity of 0.927 % LA against 0.842 % LA for control achieved similar acidity levels after 10 weeks of storage.

Incipient lactic acid development in fermented products during storage has been attributed to the unarrested cellular enzyme activity of the starter microorganisms, even though their metabolic activity is considerably reduced during storage (Choi and Kosikowski 1985). Carbon dioxide inhibits such acidification by interfering with the internal enzymatic equilibria; this function being enhanced due to the increased gas solubility under refrigeration temperatures. Statistical analysis of the data (Table 2) revealed that both carbonation and storage period significantly influenced the development of acidity in the beverage.

The FFA content and soluble N_2 profiles of the carbonated and control samples during storage are presented in Fig. 1(b). The samples recorded similar FFA contents till the 3rd week of storage, thereafter control samples recorded higher values than the carbonated samples. After 5 weeks of storage, control samples reported FFA content of 1.25 meq / L, while the

Table 1 Sensory scores for uncarbonated (control) & carbonated (sample) fermented dairy drink during refrigerated storage at 7 °C

Weeks of storage	Colour & appearance			Flavour			Body & texture			Overall acceptance		
	Control	Carbonated sample	P _T [#]	Control	Carbonated sample	P _T [#]	Control	Carbonated sample	P _T [#]	Control	Carbonated sample	P _T [#]
0	8.2±0.10 ^a	8.1±0.08 ^a	0.437	8.0±0.15 ^a	7.9±0.14 ^{a,b}	<0.001	8.0±0.15 ^a	7.9±0.10 ^a	<0.001	8.0±0.12 ^a	7.8±0.10 ^a	<0.001
1	7.9±0.13 ^{a,b}	8.1±0.08 ^a	<0.001	7.9±0.11 ^a	7.9±0.10 ^a	<0.001	8.0±0.10 ^a	7.8±0.10 ^{a,b}	<0.001	8.0±0.10 ^a	7.8±0.13 ^{a,b}	<0.001
2	7.9±0.09 ^b	8.0±0.05 ^a	HD	7.9±0.08 ^a	7.6±0.10 ^b	<0.001	7.9±0.06 ^a	7.8±0.14 ^{a,b}	HD	7.8±0.10 ^a	7.8±0.14 ^a	0.023
3	7.8±0.15 ^b	7.9±0.10 ^b	0.235	7.9±0.10 ^a	7.5±0.12 ^{b,c}	<0.001	7.8±0.13 ^b	7.8±0.14 ^{a,b}	0.779	7.7±0.13 ^a	7.7±0.13 ^a	0.051
4	7.8±0.16 ^b	7.8±0.10 ^b	HD	6.7±0.16 ^b	7.4±0.13 ^{b,c}	<0.001	7.7±0.14 ^b	7.7±0.16 ^{a,b}	0.056	6.7±0.16 ^b	7.7±0.17 ^a	<0.001
5	7.8±0.16 ^b	7.8±0.14 ^b	0.725	5.1±0.39 ^c	7.4±0.11 ^{b,c,d}	HD	7.5±0.15 ^c	7.6±0.17 ^{b,c}	0.044	5.1±0.39 ^c	7.5±0.15 ^{b,c}	HD
6	–	7.8±0.08 ^b	–	–	7.3±0.10 ^{c,d}	–	–	7.6±0.20 ^{b,c}	–	–	7.3±0.10 ^{c,d}	–
7	–	7.8±0.10 ^c	–	–	7.2±0.13 ^{c,d,e}	–	–	7.5±0.10 ^{b,c}	–	–	7.3±0.13 ^{c,d}	–
8	–	7.8±0.13 ^c	–	–	7.2±0.07 ^{d,e}	–	–	7.5±0.13 ^{b,c}	–	–	7.6±0.14 ^{c,d}	–
9	–	7.7±0.09 ^c	–	–	7.0±0.10 ^{e,f}	–	–	7.5±0.08 ^{b,c}	–	–	7.3±0.20 ^{c,d}	–
10	–	7.6±0.10 ^d	–	–	7.0±0.08 ^{e,f}	–	–	7.2±0.24 ^c	–	–	7.0±0.10 ^d	–
11	–	7.3±0.17 ^e	–	–	6.8±0.14 ^f	–	–	7.1±0.10 ^{c,d}	–	–	7.0±0.17 ^d	–
12	–	7.2±0.13 ^e	–	–	6.0±0.13 ^g	–	–	6.7±0.23 ^d	–	–	6.6±0.21 ^e	–
P _{SP} [#]	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

Value presented are means ± SD of 30 observations

a parameter is significant only when $p < 0.05$, means sharing the same superscript in a column are not significantly different from each other (Tukey's HSD, $p < 0.05$); P_T - p value for one way ANOVA (between treatment), P_{SP} - value for one way ANOVA (between storage period), HD- Heteroscedastic data

Fig. 1 Variation in chemical characteristics of the carbonated fermented dairy drink and control during storage at 7 °C. Values are mean $\pm$ SD of 3 replications. (LA: lactic acid, FFA: free fatty acid)

carbonated samples recorded a value of 0.886 meq / L. The occurrence of lipolysis in cultured / fermented milk products is often quantified as an increase in FFA content (Ashwani et al 2003a; Behare and Prajapati 2007). Lipolysis in dairy products

is attributed to the activity of either endogenous lipoprotein lipase in bovine milk fat (Deeth et al 1975; Castberg 1992) or lipolytic enzymes of microbial origin and other miscellaneous esterases (Azzara and Dimick 1985).

The effect of carbonation on the soluble nitrogen content became evident after 3 weeks of storage; wherein the control sample showed significantly higher soluble nitrogen content than that of the carbonated samples (Fig. 1b). Soluble nitrogen can be used as an index of proteolysis in fermented milk products (Ashwani et al 2003b; Behare and Prajapati 2007). The effect of dissolved CO₂ in retarding the development of proteolysis and lipolysis in pasteurized and fermented milks has been reported in Hotchkiss et al (2006) and Choi and Kosikowski (1985). Proteolytic activity in milk may be due to the enzyme plasmin (de Rham and Andrews 1982), non-plasmin proteolytic activity is anticipated only when there is a significant population of somatic cells (Verdi and Barbano 1991). Ma et al (2003) reported the influence of reduced pH in milk in inhibiting plasmin activity. They attributed the reduced plasmin activity to it being an alkaline serine protease with maximum activity at higher pH. Choi and Kosikowski (1985) discussed the influence of yeast and mold growth on the development of proteolysis in yoghurt beverages.

Thus, the reduced proteolysis (soluble N₂) and lipolysis (FFA) exhibited by the carbonated samples during storage could be attributed to inhibited microbial growth due to the dissolved CO₂. Statistical analysis of the data revealed that though initially there was no significant difference between the treatments; carbonation significantly influenced the FFA and soluble N₂ content of the samples after 6 weeks of storage. The storage period significantly influenced the FFA and soluble N₂ content in both control and carbonated samples.

Changes in microbiological quality

The microbiological profile of the beverage samples during storage is depicted in Fig. 2. The SPC of the control and carbonated beverage was found to progressively diminish during storage. This trend is consistent to that reported for fermented milks and is ascribed to the outgrowth of lactic acid bacteria by yeast and molds in the presence of highly acidic environment (Hussain et al 2012). It was observed

Table 2 Statistical analysis of the effects of storage period and treatment on chemical and microbial attributes of fermented dairy drink

Parameter	F Ratio ^a					
	pH	Acidity	FFA	Soluble N ₂	LN(SPC)	LN(YMC)
Treatment (control / carbonated)	3.759	2.003	6.707*	0.858	4.253*	0.339
Storage Period	42.428***	9.303***	20.910***	8.394***	20.091***	25.888***
Treatment $\times$ Storage period	44.069***	32.318***	16.880***	68.706***	40.375***	117.986***

^a a parameter is significant only when * p <0.05, *** p <0.0001

Fig. 2 Changes in microbial profile of carbonated fermented dairy drink and control during storage at 7 °C. Values are mean $\pm$ SD of 3 replications. (SPC: Standard Plate Count, YMC: Yeast and Mold count)

that the control samples had a higher SPC after one week of storage; thereafter the SPC of the control was slightly lower than that of carbonated samples. However, the presence of dissolved carbon dioxide in the beverage was found to significantly inhibit the growth of yeasts and molds in the sample. This is important since yeasts are a major cause of spoilage of yogurt and fermented milks in which the low pH provides a selective environment for their growth (Ledenbach and Marshall 2009)

Karagul-Yuceer et al (2001) studied the effect of dissolved CO₂ in yoghurt and reported that carbonation did not affect the bacterial population (both desirable and spoilage microbes) in yogurt. An earlier study conducted by Choi and Kosikowski (1985) reported that the yeast and mold growth in carbonated yoghurt beverage was suppressed due to carbon dioxide, while the growth rate of lactic acid bacteria was unaffected by carbonation. The authors attributed this result to “selective bacterial inhibition phenomena” of carbon dioxide. Statistical analysis of the data revealed that the microbial profile of the sample was influenced by the storage period and carbonation.

Conclusion

Carbonation at 50 psi for 30 s for fermented dairy beverage (*lassi*) was observed to have no adverse impact on the sensory quality of the product. Carbonation did not significantly alter

the pH of the product while registering a marginal increase in the titratable acidity of the product. Dissolved carbon dioxide aided in arresting the development of lipolysis and proteolysis and inhibiting the growth of yeasts and molds in the product during storage. The carbonated samples were found to be acceptable up to 12 weeks while the un-carbonated control sample kept well up to 5 weeks under refrigerated storage. Dissolved CO₂ could be used as a simple and inexpensive processing aid to extend shelf life of fermented dairy products.

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