Extended shelf life flavoured dairy drink using dissolved carbon dioxide

Menon Rekha Ravindra · K. Jayaraj Rao ·
B. Surendra Nath · Chand Ram

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Abstract Cardamom flavoured dairy beverage prepared using standardized method was carbonated in glass bottles. Carbonation at 50 psi pressure for 30 s was recommended. The pasteurized flavoured drink, carbonated or otherwise was evaluated for sensory, chemical and microbial quality during its refrigerated storage. The uncarbonated control samples were found to be sensorily acceptable up to 14 days, while the carbonated beverage remained acceptable up to 30 days. Carbonation of drink significantly affected the pH and acidity of product without reducing its acceptability. Carbonation resulted in inhibition of microbes, the effect was pronounced on psychrotrophic count. There was a linear but marginal increase in the pH of the carbonated samples till the 17th day of storage; the values diminished thereafter. The carbonated samples also had significantly reduced contents of FFA and soluble nitrogen compared to that of uncarbonated control samples as storage progressed beyond 10 days and this was attributed to inhibited microbial growth.

Keywords Flavoured milk · Carbonation · Sensory · Chemical · Microbial · Shelf life

Introduction

Flavoured milk is a sweetened milk beverage, usually made using low fat milk, sugar, permitted colourings and artificial or natural flavourings. Flavoured milk, in the Indian market, has a projected annual growth rate of 27% (Baisya

2005). The flavoured milk market in India is dominated by popular brands like Amul, Britannia and Nestle and the major regional brands include Saras in Rajasthan, Verka in Punjab and Nandini in Karnataka. Flavoured milk is usually pasteurized, sterilized or heat treated at ultra-high-temperature for extending its shelf life. The pasteurised flavoured milk has a shelf life of 5–7 days under refrigerated storage while the sterilized product has a shelf life of about 90–180 days at room temperature. For extended shelf life, apart from heat sterilisation, local manufacturers use other techniques like chilled storage.

Carbon dioxide (CO₂) has a prevailing inhibitory effect on microbial growth (Dixon and Kell 1989) and has been used as a means for extending the shelf life of foods in technologies like controlled atmosphere and modified atmosphere packaging (Kader 1986). Carbonation is practiced in many beverages, like popular soft drinks, for the purpose of imparting a unique sparkle or effervescence, flavor enhancement, and a refreshing aftertaste. The “fizz” generated in the beverage due to dissolved CO₂ could position the dairy beverage as a novel and nutritive beverage, appealing to the younger generation as a healthy alternative to carbonated soft drinks.

The antimicrobial action of dissolved CO₂ can be attributed to several factors. Daniels et al. (1985) proposed that the displacement of oxygen by CO₂ in the product may contribute to the slowing down of the growth rate of aerobic bacteria. Hydration of CO₂ to carbonic acid may result in a reduced pH creating a stress in the microbial environment influencing its physiological activity adversely (Wolfe 1980). Dixon et al. (1987) postulated that CO₂ could interfere in biochemical pathways resulting in reduced microbial growth. The role of CO₂ in alteration and regulation of enzymes, thereby inhibiting microbial activity has also been reported (Pichard et al. 1984).

M. R. Ravindra (✉) · K. J. Rao · B. S. Nath · C. Ram
National Dairy Research Institute, Southern Campus, Adugodi,
Bangalore India 560 030
e-mail: rekhamn@gmail.com

Since dissolved CO₂ is a natural component of freshly drawn milk that is subsequently lost during processing and transit, there is an ample scope for its utilization as a simple, inexpensive technique to extend shelf life of dairy products. This technique has already been reported for extension of shelf life of milk (Hotchkiss and Lee 1996) 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 flavoured dairy drink and its effect on the chemical and microbial quality of the beverage.

Materials and methods

Preparation of Flavoured Dairy Drink (FDD)

Raw milk procured from the Experimental Dairy Plant of the Institute was standardized to 1.5% fat and 8.5% SNF. The milk was homogenized at 65 °C in a two-stage APV Crepaco homogeniser operated at 1,500/500 psi and mixed with sugar at 10% (selected based on preliminary sensory investigations). The drink was pasteurized at 85 °C for 20 min, followed by cooling to 15 °C. Preliminary studies were conducted to select a suitable flavour; the flavours studied included conventional flavours (cardamom and rose) and non-conventional flavours (orange and pineapple). The level of various flavours and colours used for selection of the appropriate flavour for the carbonated FDD is outlined in Table 1. Based on these preliminary studies, cardamom was selected as the flavour for the beverage. The flavoured dairy drink was filled into sanitised glass bottles of 200 ml capacity for carbonation and storage.

Carbonation procedure

A domestic carbonator (Mr. Butlers Instafizz, Kerala) was modified by fitting it to a 1 kg CO₂ gas cylinder with necessary attachments of regulators and pressure gauges. This system was utilized for injecting CO₂ gas into the beverage samples. The system was leak- tested before each trial using the standard soap bubble test. The glass bottles containing FDD was placed on the platform in such a manner that the gas injection pipe remained dipped in the sample during carbonation. The platform lever was operated to raise the bottle 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 3–4 days intervals. The sensory quality of the product and control was judged using a 9-point hedonic scale (BIS 1971).

Physico-chemical and microbial analysis

The proximate composition of the milk used for the study was determined using standard procedures (BIS 1981). The physico-chemical attributes of flavored dairy drink were evaluated for each sample during storage. pH (Potentiometric method, Systronics digital pH meter: MK–VI) and titratable acidity (BIS 1960) of the samples were determined for both the carbonated and uncarbonated samples. The effect of carbonation on lipolysis and proteolysis, if any, 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 its Total Plate Count (TPC) and Psychrotrophic Plate Count (PPC). The former was determined from nutrient agar plates incubated at 37 °C for 48 h while PPC was enumerated from milk agar plates incubated at 7 °C for 10 days both plates being diluted to appropriate decimal levels (Lutgring et al. 1997).

Statistical analysis

The effect of carbonation and storage period on the sensory, chemical and microbial profile of the stored beverage was analysed by two- factor ANOVA, using SYSTAT 8.0. The experiments were conducted using a fully factorial design of 2×10×3 for 2 treatments (with and without carbonation) and 10 storage periods in triplicate.

Results and discussion

Selection of gas pressure—injection time for carbonation

The proximate composition of the milk used for the study was observed to be fat 1.5%, protein 3.2%, lactose 4.6% and ash 0.68%. The cardamom flavoured milk beverage was prepared using the levels of colour and flavour as outlined in Table 1. The beverage was carbonated at different combinations of injection pressure (50–90 psi, at increments of 10 psi) and time (20–90 s, at increments of 10 s) and the sensory threshold for the degree of

Table 1 Levels of added colours and flavours for the preparation of carbonated flavored dairy drink

Flavour	Ingredient description	Source	Usage level
Orange	Colour–1% Orange red	Bush Boake Allen (India) Ltd. IH 7802	6 ml/L
	Flavour–Sweet Orange No 1	Bush Boake Allen (India) Ltd.	5 ml/L
Rose	Colour–1% Raspberry Red	Bush Boake Allen (India) Ltd. IH 7804	3 ml/L
	Flavour–Rose essence	Aromatik Bangalore	1 ml/L
Pineapple	Colour–1% Lemon Yellow	Bush Boake Allen (India) Ltd. IH 6597	10 ml/L
	Flavour–Pineapple No 1	Bush Boake Allen (India) Ltd.	
Cardamom	Colour–1% Lemon Yellow	Bush Boake Allen (India) Ltd. IH 6597	5 ml/L
	Ground Cardamom seeds ^a	Local Market	3 g/L

^a the sediment was filtered during bottling

carbonation was evaluated subjectively. The corresponding pH of the carbonated beverage was also recorded. From the investigation, it was deduced that the pressure–time combination resulting in a pH above 6.0 was an acceptable degree of carbonation. When the pH of the drink dropped below 6.0, slight tanginess was perceived rendering the drink unacceptable. A pressure–time combination of 50 psi and 30 s (for a sample size of 200 ml) was selected for carbonating the beverage at 15 °C. Carbonation at 15 °C ensured that the drink was not saturated with CO₂ at the storage temperature of 7 °C and all the injected gas remained dissolved in the drink.

Changes in the quality of FDD during storage

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

Changes in sensory quality

The sensory scores obtained for the experimental samples and control during storage are presented in Table 2. The carbonated beverage scored marginally lower than the control sample for flavour, consistency and overall acceptability due to very slight fizz (expressed as “just detectable”) experienced by the sensory panel. During the first week there was no change in the sensory attributes of both the control and the sample as reflected by the steady scores during this period. The samples, however, were found to lose freshness and hence were observed to obtain diminishing scores for flavour. No appreciable change in the viscosity of the drink was observed during storage and this was reflected in the consistency scores of the samples during sensory evaluation. On the 17th day, the control sample was observed to have developed an acidic flavour, with onset of curdling and was rejected during sensory evaluation.

Table 2 Effects of carbonation and storage period on various attributes of flavoured dairy drink

Days of storage	Colour & appearance		Flavour		Consistency		Overall acceptance	
	Control	Carbonated sample	Control	Carbonated sample	Control	Carbonated sample	Control	Carbonated sample
0	8.3	8.3	8.1	7.6	8.0	7.8	8.1	7.7
3	8.3	8.3	8.1	7.6	8.0	7.8	8.1	7.7
7	8.2	8.2	8.1	7.6	8.0	7.8	8.1	7.7
10	6.6	8.0	7.6	7.3	7.8	7.8	7.4	7.6
14	6.5	8.0	7.5	7.3	7.5	7.8	7.4	7.5
17	5.7	7.7	2.0	7.3	3.3	7.8	2.3	7.5
20	–	7.7	–	7.3	–	7.7	–	7.3
24	–	7.5	–	7.1	–	7.5	–	7.0
27	–	6.2	–	7.1	–	7.5	–	7.0
30	–	5.5	–	4.0	–	6.1	–	4.2

Values presented are average of triplicate scores

The pH of the carbonated sample fell below 6.0 after 24 days of storage and this was reflected in its poorer flavour scores. However, the sensory scores indicated that the samples were still acceptable. This was in contrast to our observations during the preliminary studies to select carbonation levels where a pH below 6.0 was deemed unacceptable by the sensory panel. Hence, it could be deduced that the decrease in pH below 6.0 during storage was not due to carbonation effect and its resultant tanginess but may be attributable to microbial activity.

The carbonated sample scored poorly by the 4th week and was rejected sensorily by the 30th day. Thus, a near two-fold increase in shelf life of the beverage was achieved by carbonation. The results of statistical analysis of the sensory scores are summarized in Table 3. It was observed that while the scores for consistency were not influenced by the carbonation treatment or storage period, colour and appearance was influenced by both the factors. The scores for flavour and overall acceptance were significantly influenced by the storage period.

Changes in physiochemical quality

The effect of carbonation on the chemical attributes of the flavored drink evaluated during storage is presented in Figs. 1 and 2. The injection of carbon dioxide into the milk beverage was observed to decrease the pH of the product, possibly due to the dissolution of the gas and the resultant formation of carbonic acid (Rajagopal et al. 2005). This could also be the reason for the higher acidity of the carbonated sample in comparison with the acidity of the control. The increased acidity attributed to the dissolved CO₂, however, did not affect the stability of the carbonated samples as is reflected from its acceptable sensory scores for consistency. On storage, the control sample progressively became acidic resulting in an increased acidity value. There was a linear but marginal increase in the pH of the

carbonated samples till the 17th day of storage, pH values diminished thereafter. The acidity of the carbonated sample showed only a marginal increase till 14 days of storage; thereafter the samples recorded progressively increasing acidity values but this did not significantly affect the flavour scores during sensory evaluation (Table 2). The effect of carbonation and storage on the pH and acidity of drinks was statistically significant (Table 3)

The FFA content and soluble N₂ profile of the samples during storage are presented in Fig. 2. The FFA contents of the carbonated and control samples almost paralleled each other till about 10 days of storage; thereafter the FFA content of the control sample recorded higher values than that of the carbonated samples. The control samples also recorded a significant increase in soluble N₂ content after 10 days of storage. Statistical analysis of the data revealed that the storage period and treatment had significant influence on FFA content of the samples. Statistically, the soluble N₂ content of the samples was also significantly influenced by the storage period and carbonation.

Increase in FFA in milk has been used as an index of lipolysis (Ma et al. 2003), which is attributed to the activity of either the native lipoprotein lipase in bovine milk (Castberg 1992) or other lipolytic enzymes of somatic cells and bacteria and other miscellaneous esterases (Azzara and Dimick 1985). In practice, rancid flavour is detectable when the FFA value of milk exceeds 2 meq/L (Brathen 1980). The values reported for the samples for this study were within this limit and no rancid, soapy or bitter flavour was reported during sensory evaluation, eliminating the possibility of hydrolytic rancidity.

Similarly, soluble nitrogen is used as an index to proteolysis (Stone and Naff 1967). Most of the proteolytic activity in normal milk is attributed to the enzyme plasmin (de Rham and Andrews 1982). Ma et al. (2003) reported the decreased proteolysis of carbonated raw milk and attributed this effect to reduced microbial proteases and

Table 3 Statistical analysis of the effects of storage period and treatment on various attributes of flavoured dairy drink

Sensory property	<i>p</i> values ^a			
	Colour & appearance	Flavour	Body & texture	Overall acceptance
Storage period, (days)	<0.0001	0.0297	0.0615	0.0182
Treatment, (carbonated/uncarbonated)	0.0124	0.3248	0.1568	0.2285
Physio-chemical property	pH	Acidity	FFA	Soluble N ₂
Storage period, (days)	0.0020	<0.0001	<0.0001	0.0013
Treatment, (carbonated/uncarbonated)	0.0254	0.0045	0.0473	0.0310
Microbiological property	TPC	PPC		
Storage period, (days)	<0.0001	0.2598		
Treatment, (carbonated/uncarbonated)	0.4584	0.0001		

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

Fig. 1 Variation in pH and acidity of the carbonated flavoured dairy drink and control during refrigerated storage

the possible reduced endogenous protease activity due to a reduced pH. Cousin (1982) discussed the effect of extra cellular enzymes produced by psychrotrophic bacteria on proteolytic and lipolytic changes in milk. Thus, the reduced proteolysis (soluble N_2) and lypolysis (FFA) exhibited by the carbonated samples could be attributed to inhibited microbial growth.

Changes in microbiological quality

The microbiological profile of the beverage samples during storage is depicted in Fig. 3. The initial total viable count for the drink was $4.39 \pm 0.09 \log_{10}$ cfu/ml. Carbonation of the beverage inhibited the growth of microbes; this was more so in case of psychrotrophic microorganisms (Fig. 3). The sensitivity of psychrotrophs to dissolved CO_2 in milk was previously reported by King and Mabbitt (1982). The

Fig. 3 Changes in microbial profile of carbonated flavoured dairy drink and control during refrigerated storage. (TPC: Total Plate Count, PPC: Psychrotrophic Plate Count)

antimicrobial effect of dissolved CO_2 observed in this study is consistent with previous reports (Hotchkiss and Lee 1996; King and Mabbitt 1982). Statistical analysis of the data revealed that for TPC, the storage period was an influential parameter, with no significant difference due to carbonation. In the case of PPC, carbonation had a significant effect, while the storage period was not significant.

Conclusion

Carbonation at 50 psi for 30 s for 200 ml of pasteurized flavoured dairy drink packaged in glass bottles is recommended. The carbonation of drink had a significant effect on raising the acidity and lowering the pH of system without adversely affecting the sensory quality of product. The carbonation inhibited the growth of microbes, especially psychrotrophs, during storage, nearly doubling the shelf life (up to 30 days vs. 17 days for control) of beverage when stored under refrigeration. During its refrigerated storage, the carbonated drink exhibited lower changes in FFA and soluble nitrogen content than the control. The antimicrobial properties of CO_2 could be used as a simple, inexpensive processing aid to extend shelf life of flavoured dairy beverage.

Fig. 2 Variation in soluble N_2 and FFA of the carbonated flavoured dairy drink and control during refrigerated storage

References

- Azzara CD, Dimick PS (1985) Lipolytic enzymes activity of macrophages in bovine mammary gland secretions. *J Dairy Sci* 68 (7):1804–1812
- Baisya RK (2005) Value addition in dairy industry–vision 2020. Souvenir of National Seminar on value added dairy products. 21–22 Dec, National Dairy Research Institute, Karnal, 1–4
- BIS (1960) Method for test for dairy industry, part-I: rapid examination of milk. IS: 1479. Bureau of Indian Standards, New Delhi
- BIS (1971) Guide for sensory evaluation of foods, part-II: methods and evaluation cards, IS: 6273. Bureau of Indian Standards, New Delhi
- BIS (1981) Handbook of analysis of food products, part-XI: dairy products, SP18. Bureau of Indian Standards, New Delhi
- Brathen G (1980) Lipolysis in milk: automated determination of the acidity value with the autoanalyzer, and assessment of results. *Meieriposten* 69(13):345–352
- Castberg HG (1992) Lipase activity. IDF bulletin no. 271: Int. Dairy Fed., Brussels, Belgium 18–20
- Cousin MA (1982) Presence and activity of psychrotrophic microorganisms in milk and dairy products: a review. *J Food Prot* 45:172–207
- Daniels JA, Krishnamurthi R, Rizvi SSH (1985) A review of effects of carbon dioxide on microbial growth and food quality. *J Food Prot* 48(6):532–537
- de Rham O, Andrews AT (1982) Qualitative and quantitative determination of proteolysis in mastitic milks. *J Dairy Res* 49 (4):587–596
- Deeth HC, Fitz-Gerald CH, Wood AF (1975) A convenient method for measuring the extent of lipolysis in milk. *Aust J Dairy Tech* 30 (3):109–110
- Dixon NM, Kell DB (1989) The inhibition by carbon dioxide of the growth and metabolism of microorganisms. *J Appl Bacteriol* 67 (2):109–136
- Dixon NM, Lovitt RW, Kell DB, Morris JG (1987) Effects of pCO₂ on the growth and metabolism of *Clostridium sporogenes* NCIB 8053 in defined media. *J Appl Bacteriol* 63(2):171–182
- Hotchkiss JH, Chen JH (1996) Microbiological effects of the direct addition of CO₂ to pasteurized milk. *J Dairy Sci* 79(suppl 1):87
- Hotchkiss JH, Lee E (1996) Extending shelf-life of dairy products with dissolved carbon dioxide. *Eur Dairy Mag* 8(3):18–19
- Kader AA (1986) Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables. *Food Tech* 40(5):99–104
- Karagul-Yuceer Y, Coggins PC, Wilson JC, White CH (1999) Carbonated yogurt–sensory properties and consumer acceptance. *J Dairy Sci* 82(7):1394–1398
- King J, Mabbitt LA (1982) Preservation of raw milk by the addition of carbon dioxide. *J Dairy Res* 49(3):439–447
- Lutgring KR, Linton RH, Zimmerman NJ, Peugh M, Heber AJ (1997) Distribution and quantification of bioaerosols in poultry–slaughtering plants. *J Food Prot* 60(7):804–810
- Ma Y, Barbano DM, Santos M (2003) Effect of CO₂ addition to raw milk on proteolysis and lipolysis at 4 °C. *J Dairy Sci* 86(5):1616–1631
- Mann EJ (1991) Cottage cheese. *Dairy Indust Int* 56(6):14–15
- Pichard B, Simard RE, Zee JA, Bouchard C (1984) Effect of nitrogen, carbon monoxide, carbon dioxide on the activity of proteases on *Pseudomonas fragi* and *Streptomyces caespitosus*. *Sci Aliment* 4 (4):595–608
- Rajagopal M, Werner BG, Hotchkiss JH (2005) Low pressure CO₂ storage of raw milk: microbiological effects. *J Dairy Sci* 88 (9):3130–3138
- Rowland SJ (1938) The determination of the nitrogen distribution in milk. *J Dairy Res* 9:42–46
- Stone WK, Naff DM (1967) Increases in soluble nitrogen and bitter flavor development in cottage cheese. *J Dairy Sci* 50(9):1497–1500
- Wolfe SK (1980) Use of carbon monoxide and carbon dioxide enriched atmospheres for meats, fish and produce. *Food Tech* 34(3):55–58