Effect of heat treatment on the storage stability of low calorie milk drinks

Shikha Mittal · Usha Bajwa

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Abstract The study was undertaken to study the effect of heat treatment on the storage stability of cardamom flavoured low calorie milk drinks (CFDs). The drinks prepared by replacing sugar with sucralose and adding inulin in milk of 0.5 % fat and 8.5 % milk solid-not-fat were subjected to pasteurization and sterilization and stored at refrigeration and room temperature, respectively. The stored samples were evaluated for changes in physico-chemical and sensory attributes at regular intervals. In pasteurized drinks, the total solids (TS) and pH declined while the total soluble solids (TSS), titratable acidity and viscosity increased significantly ($p < 0.01$) with storage. A significant reduction in the flavour and body and mouthfeel scores was observed. Standard plate count (SPC) increased in both control and low calorie drinks with storage period. In sterilized CFDs, TS and TSS were not affected appreciably whereas titratable acidity increased and viscosity decreased significantly ($p < 0.01$) with storage. Though the sensory scores also declined with storage, the drinks obtained high acceptability scores even after 150 days of storage at room temperature. However, the changes in colour components (L, a and b values) indicated increased browning in the drinks with storage time. SPC was not detected until 120 days in control and 135 days in low calorie drink. Yeast and molds were not evident until 135 days in control and 150 days in low calorie drink. The shelf life was found to be 10 and 150 days of pasteurized and sterilized CFDs at refrigeration and room temperature, respectively.

Keywords Low calorie · Milk · Inulin · Sucralose · Physico-chemical · Viscosity · Sensory quality · Storage

Introduction

Sweeteners are important ingredients in food and have been so for centuries. In spite of having many functional properties alongwith sweetness, sugar together with fat and/or starch is cited as a culprit in attaining overweight. Sugars have also been implicated as causative agents in a number of disorders like diabetes, dental caries, hyperglycemia etc. (Arora et al. 2001). The growing search for products that offer fewer calories and more functional and nutritional qualities has contributed to a large variety of low calorie sweeteners in the world market and revolutionized the food market with diet and low calorie products (Mendonca et al. 2001).

The dairy industry has radically adapted itself to the changing scenario. In pursuit of sugar replacement, the use of high potency artificial sweeteners to develop low calorie dairy products has a high potential. Delicious milk foods and beverages can be produced by using low calorie artificial sweeteners. Use of artificial sweeteners has been allowed in sweets like *halwa*, *khoya*, *burfi*, *rasogolla*, *gulabjamun* and other milk products (Arora et al. 2006). Sucralose has been granted FDA approval as a “general purpose sweetener” (Goyal et al. 2007). Codex (2005) has allowed the use of sucralose to a maximum limit of 300 ppm in dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa), 250 ppm in dairy-based desserts e.g. ice-cream, ice milk, pudding, fruit or flavoured yoghurt. It has been found suitable for use in formulations for low calorie dairy Mousses (Porto-Pinto et al. 2003). Chocolate dessert containing sucralose had a 31 % lower calorie content.

S. Mittal · U. Bajwa (✉)
Department of Food Science and Technology,
Punjab Agricultural University,
Ludhiana 141004, Punjab, India
e-mail: ushabajwa@rediffmail.com

In keeping with the consumers' demand for foods to be low in fat, sugar and calories along with health promoting ingredients, Mittal and Bajwa (2011) developed cardamom flavoured low calorie milk drink incorporating sucralose and inulin. However, it is imperative to investigate the shelf life/storage stability of the products so developed before launching them for commercial use. Milk beverages with a longer shelf life can help meet the strain put on distribution centers and demands resulting from the consolidation of manufacturing facilities. A longer shelf life also helps reduce the return of expired products and enables the opening of new distribution channels. In general, a longer shelf life improves the marketability of slower-turning products such as milk beverages. Therefore, the objective of the investigation was to study the effect of heat treatment on the shelf stability of low calorie milk drinks. This was envisaged by investigating physico-chemical and microbiological changes occurring during storage at room and refrigeration temperatures.

Materials and methods

Milk was procured from Dairy Processing Plant of the university. It was separated in a cream separator (Elecrem, France) to obtain skim milk, blended with the standardized milk (4.5 % fat) to achieve fat content of 2.0 and 0.5 % for control drink and low calorie drink, respectively. The milk solids-not-fat (MSNF) was standardized to 8.5 % using skim milk powder (Verka brand, Ludhiana, India). Long chain inulin (Beneo ST, degree of polymerization >10) of *Orafti – active food ingredients*, was a dried, amorphous powder, procured from DPO Food Specialities Pvt. Ltd, Mumbai. Sugar and sucralose (*Splenda*) were purchased from local market. The sucralose tablets were powdered for use in low calorie samples. Based on preliminary trials 6 % sucrose was used in control drinks and 660 mg/L *Splenda* equivalent to 72.6 ppm sucralose in low calorie samples (Mittal and Bajwa 2011). The process of preparation of cardamom flavoured milk drinks (CFD) is presented in Fig. 1. After standardization of milk fat, to control drink 6 % sugar and to low calorie drink 660 mg/L *Splenda* (72.6 ppm sucralose) powder and 4 % inulin were supplemented. Cardamom powder (0.2 %) was added in the drinks, heated to 65 °C and properly stirred to dissolve sugar or sucralose and inulin. The contents were filtered, homogenized, filled in sterilized glass bottles (200 ml) and sealed. The filled bottles were subjected to two heat treatments. In the first, bottles were pasteurized (75 °C for 15 s), cooled and stored at 5.0±0.1 °C. In the second, bottles were sterilized at 116 °C for 15 min, cooled and stored at room temperature (34.1±0.3 °C).

Physicochemical analyses For each parameter, the samples were analysed in three replications. Total soluble solids

Fig. 1 Flow diagram for preparation of cardamom flavoured milk drinks. (MSNF: milk solid-not-fat)

(TSS°B) of milk drink samples were determined at 20 °C using Hand refractometer (Erma, Japan). The pH of all the samples was determined using pocket pH meter (Model IQ 125, IQ Scientific, USA). The titratable acidity of milk and milk drinks was determined by titrating 10 ml of sample against 0.1 N NaOH and expressed as % lactic acid. Lane and Eynon method of Ranganna (1994) was used for determining reducing sugars in milk drink samples. The method was slightly modified for the determination of total sugars. Dynamic viscosity in centipoise (cP) of all the milk drinks was measured using the Hoppler Viscosimeter (design type BH2, Surrey, England) at 20 °C. The time of fall of a ball (No.1) in a cylindrical tube (internal diameter=15.94 mm) inclined by 10 ° with respect to the vertical plane and filled with the liquid to be examined, was measured. The time taken by the ball to travel a distance of 100 mm was recorded. The dynamic viscosity was calculated as per Mittal and Bajwa (2011). Colour of milk drinks was measured using Mini scan Xe Plus (Hunter Colour Lab) in the Hunter Colour mode and expressed as 'L', 'a' and 'b' values.

Sensory evaluation All the milk drink samples were evaluated for appearance/colour, flavour, consistency and mouth-feel and overall acceptability by 8 semi-trained panelists from the department using a 9-point hedonic scale (Meilgaard et al. 1999) with scores ranging from liked extremely (9) to disliked extremely (1).

Microbiological studies Standard plate count (SPC) and yeast and mould count (YMC) were recorded as per APHA (1984) procedures using nutrient agar and glucose yeast extract agar, respectively.

Statistical analysis For each parameter, the samples were analysed in three replications. The experimental data was

subjected to analysis of variance (Snedecor and Cochran 1968) using CPCS1 software developed by the Department of Mathematics, Statistics and Physics of the university.

Results and discussion

Changes in physico-chemical characteristics of the pasteurized drinks during storage TS content of the drinks decreased and TSS increased significantly ($p<0.01$) with progress in storage. It may be due to the hydrolysis of components. Control drink had more TSS than low calorie drink due to presence of sugar. Acidity increased significantly ($p<0.01$) during storage. The rate of increase was more in control as compared to low calorie drink. The pH of both the drinks decreased significantly ($p<0.01$) with storage showing higher decline in control (Table 1). Both the samples kept well up to 10 days. Thereafter, a higher acidity was noted in control as compared to low calorie drink resulting

in flaky and curdled appearance. Added sucrose in the control drink acted as a source of microbial spores which might have germinated during storage and led to its faster spoilage. Pasteurization of milk kills the more active acid-forming bacteria but permits the survival of heat resistant lactics. Moreover, spoilage problems of pasteurized milk at refrigeration temperature are related to spore-forming psychrotrophic bacilli (Frazier and Westhoff 2003).

The reducing sugars (RS) in control declined whereas in low calorie drink, it increased. The difference in RS was not significant with storage. Added sugar may be a source of microbial spores in control drink. These spores along with the available microflora converted the RS into acid during storage, causing a decline in RS content in control. However, in low calorie drink, inulin (a fructo-oligosaccharide, FOS) acted as prebiotic for the microflora which partly broke it into constituent fructan units and resulted in overall increase in the RS content during storage (Silva 1996). Shin et al. (2000) found that growth promotion, enhancement of

Table 1 Changes in physico-chemical characteristics of the cardamom flavoured pasteurized milk drinks during storage

Type of drink	Storage (5.0 ± 0.1 °C), days						
	0	2	4	6	8	10	12
Total solids, %							
Control	16.5	16.5	16.5	16.4	16.4	16.2	16.1
Low calorie	13.2	13.2	13.1	13.1	12.9	12.8	12.7
Total soluble solids (TSS), °B							
Control	13.9	13.9	14.0	14.0	14.2	14.3	14.1
Low calorie	10.9	11.0	11.0	11.2	11.2	11.4	11.5
Titrateable acidity, % Lactic acid							
Control	0.14	0.14	0.15	0.16	0.18	0.19	0.21
Low calorie	0.14	0.14	0.15	0.16	0.17	0.18	0.20
pH							
Control	6.6	6.6	6.5	6.3	6.2	6.0	5.9
Low calorie	6.6	6.6	6.5	6.4	6.3	6.2	6.0
Reducing sugar, %							
Control	4.3	4.3	4.3	4.2	4.1	3.9	3.9
Low calorie	4.4	4.4	4.4	4.4	4.4	4.5	4.5
Viscosity, cP $\times 10^{-2}$							
Control	280.2	280.6	281.8	283.5	287.2	291.8	300.2
Low calorie	273.3	278.7	285.4	291.3	292.7	297.1	317.5
ANOVA							
Source	df	MSS					
		Total Solids	TSS	Acidity	pH	Reducing Sugars	Viscosity
Storage (S)	6	0.17**	0.19**	0.003**	0.37**	0.024	0.02**
Drink type (D)	1	118.9**	88.1**	0.0005	0.69**	0.80**	0.02**
S X D	6	0.004	0.031	0.0001	0.01**	0.08	0.009**
Error	28	0.024	0.021	0.0002	0.002	0.035	0.0001

** Significant at $p<0.01$, ($n=3$), ANOVA- Analysis of variance, *df*-degree of freedom, *MSS*- mean sum of squares

activity and retention of viability of *Bifidobacterium* spp were higher when it was grown in 12 % (w/v) reconstituted non-fat dry milk in the presence of FOS particularly inulin.

The viscosity of control and low calorie drink increased significantly during storage (Table 1). The rate of increase in viscosity was lower in control due to higher fat content while in low calorie sample the rate of increment was higher due to increased reaction between inulin, protein and water to form gel. High viscosities on the 12th day might be attributed to the coagulation of proteins due to high acidity values resulting in spoilage of drinks. Similar trend was noticed by Cano-Ruiz and Richter (1998) with carrageenan in milk drinks. They reported an increase in viscosity with storage time when milk drinks were stored at 4 °C.

Changes in sensory scores of the pasteurized drinks during storage Appearance scores did not differ significantly with storage while a significant ($p<0.01$) change was observed between the drink type i.e. control and low calorie (Table 2). The scores decreased as the storage progressed. The flavour scores of both control and low calorie drinks were reduced significantly ($p<0.01$) with storage. The reduction was prominent in control than in low calorie drink on the 12th day of storage. The increase in titratable acidity was higher in control than low calorie drink which caused a corresponding

reduction in the flavour scores. On this day of storage, both the drinks were adjudged unacceptable due to perceived fermented/acidic flavour.

Body and mouthfeel scores also declined significantly ($p<0.01$) with storage. The lowering of scores was faster in control than low calorie drink. This was due to higher titratable acidity of the milk drinks which caused noticeable thickening after 10 days of storage rendering the product unacceptable. The overall acceptability scores decreased significantly ($p<0.01$) both in control and in low calorie drink (Table 2).

Changes in microbial counts of the pasteurized drinks The standard plate count (SPC) increased with the progress in storage period. The increase was higher in control than in low calorie drink. However, SPC of both the drinks was within the safe limits up to 10 days. The SPC of pasteurized milk in its final container should not exceed 30,000 cfu/ml (BIS 1962). The yeast and mold count was detected on the fourth day of storage in control. However, in low calorie drink yeast and mold count of 1.48 log₁₀ cfu/ml was recorded after 6 days of storage. The count increased during storage in both the drinks (Table 3). This was due to the germination of psychrotrophic spores during storage. The factors that influence the stability of pasteurized milk include the quality of

Table 2 Changes in sensory scores of the cardamom flavoured pasteurized milk drinks during storage

Type of drink	Storage(5.0±0.1 °C), days						
	0	2	4	6	8	10	12
Appearance							
Control	8.5	8.4	8.3	8.3	8.2	8.2	8.2
Low calorie	8.4	8.3	8.2	8.1	8.1	8.1	8.1
Flavour							
Control	8.6	8.5	8.5	8.2	8.0	6.0	4.7
Low calorie	8.6	8.4	8.4	8.1	7.9	6.2	5.1
Body and mouthfeel							
Control	8.4	8.4	8.4	8.5	8.5	7.6	6.0
Low calorie	8.3	8.7	8.7	8.8	8.9	7.7	6.2
Overall acceptability							
Control	8.5	8.4	8.4	8.3	8.2	7.3	6.3
Low calorie	8.4	8.5	8.4	8.3	8.3	7.3	6.5
ANOVA							
Source	df	MSS					
		Appearance	Flavour	Body and mouthfeel	Overall acceptability		
Storage (S)	6	0.178	32.0**	14.1**	10.1**		
Drink type (D)	1	0.43*	0.03	1.15**	0.033		
S X D	6	0.007	0.153**	0.102*	0.023		
Error	98	0.094	0.061	0.035	0.034		

*Significant at $p<0.05$, ** Significant at $p<0.01$ ($n=8$), ANOVA- Analysis of variance, *df*-degree of freedom, *MSS*- mean sum of squares

Table 3 Changes in microbial counts of cardamom flavoured pasteurized milk drinks during storage

Type of drink	Storage(5.0±0.1 °C), days						
	0	2	4	6	8	10	12
Standard plate count, log ₁₀ cfu/ml							
Control	3.86	3.90	3.95	3.98	4.23	4.40	4.58
Low calorie	3.73	3.79	3.83	3.88	3.94	4.20	4.41
Yeast and mold count, log ₁₀ cfu/ml							
Control	ND	ND	1.48	1.85	1.94	1.98	2.26
Low calorie	ND	ND	ND	1.60	1.79	1.94	2.04
ANOVA							
Source	df	MSS		Y MC			
		SPC		Y MC			
Storage (S)	6	0.148**		1.765**			
Drink type (D)	1	0.089**		0.327**			
Error	34	0.00001		0.021			

ND: not-detectable ($n=3$) SPC-standard plate count, YMC-yeast and mold count, ANOVA- analysis of variance, df-degree of freedom, MSS- mean sum of squares

raw material, pasteurization time, resistant microorganisms to pasteurization temperatures particularly psychrotrophics, post pasteurization contaminants and storage temperature (Cromie 1991). Petrus et al. (2010) reported a shelf life of 43, 36, 8, 5, and 3 days for pasteurized milk packaged in HDPE bottles and stored at 2, 4, 9, 14 and 16 °C, respectively whereas for milk samples packaged in LDPE pouch it was estimated to be 37, 35, 7, 3, and 2 days, respectively.

Changes in physico-chemical characteristics of sterilized milk drinks during storage The TS content of control was higher than low calorie drink and it was not affected much during 150 days of storage. Similarly, TSS of control and low calorie drinks were not affected significantly during storage. However, the titratable acidity increased significantly ($p<0.01$) whereas pH showed a decline with progress in storage from 0 to 150 days (Table 4). The RS content of the control was higher than low calorie drink which was reduced after 150 days of storage (Table 4). The loss in reducing sugars of the milk drinks with progress of storage was due to their participation in Maillard reaction to form brown pigments called melanoidins. According to Meyer and Riha (2002), slight decrease in reducing sugars occurs in food products as browning occurs during storage. The viscosity values of milk drinks differed significantly ($p<0.01$) with drink type as well as storage. It decreased in both the drinks as the storage progressed. However, viscosity of low calorie drink was lower than that of control throughout the storage. Earlier, Cano-Ruiz and Richter (1998) found that sterilized milk drinks kept at room temperature showed a decline in viscosity with storage time due to increased sedimentation of proteins.

Changes in colour (L, a, b) values of sterilized milk drinks during storage “L” value which designates whiteness of the

product increased significantly ($p<0.01$) in the control and decreased in low calorie drink with storage. Increase in whiteness of control might be due to oxidation of fat in the presence of light at room temperature and thus increasing the scattering and reflection of light (Walstra and Jenness 1984). However, the darkening of low calorie drink might be attributed to increase in browning of drink upon storage.

For both the drinks, a significant increase in “a” value and decrease in “b” value was observed during storage of 150 days (Table 5). Higher magnitude of “a” represents more red colour of the product which may be interrelated with browning. This indicates that browning increased during storage of both drinks. Gothwal and Bhavadasan (1992a) also reported that in-bottle sterilized milk samples stored at room temperature showed enhanced browning intensity at the end of 150 days. But, there was not noticeable effect to cause colour defect of commercial importance. Higher ‘a’ value of low calorie drink indicated more browning in it which was due to its higher protein content. Gothwal and Bhavadasan (1992b) observed enhancement in browning due to heating of cow and buffalo milk with increase in protein and lactose level. On the other hand, there was less browning in control drink due to its higher fat content which exhibited protective effect on other components against heat induced changes through a decrease in heat transfer (Pelligrino 1994).

Changes in the sensory scores of the sterilized milk drinks during storage The change in appearance scores was non-significant up to 150 days of storage. However, it differed significantly with type of drink i.e. control and low calorie drink, in former having higher scores than latter throughout the storage period (Table 6). The flavour scores decreased considerably with storage. This decrease was due to enhanced

Table 4 Changes in physico-chemical characteristics of cardamom flavoured sterilized milk drinks during storage

Type of drink	Storage(34.1±0.3 °C), days										
	0	15	30	45	60	75	90	105	120	135	150
Total solids, %											
Control	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5	16.5
Low calorie	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.1
Total soluble solids (TSS), %											
Control	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.8	13.8	13.8
Low calorie	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.9	10.8
Titratable acidity, % Lactic acid											
Control	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16
Low calorie	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.15	0.16	0.16	0.16
pH											
Control	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.5	6.5	6.4
Low calorie	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.5	6.5	6.5	6.4
Reducing sugars, %											
Control	4.2	4.2	4.2	4.2	4.1	4.1	4.1	4.1	4.1	4.1	4.1
Low calorie	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.9	3.9	3.9	3.9
Viscosity, cP X 10 ⁻²											
Control	280.2	276.8	273.9	270.5	268.1	266.3	262.7	258.2	255.5	253.8	251.1
Low calorie	273.3	270.2	266.6	263.9	260.1	258.7	256.3	254.2	251.8	245.7	241.5
ANOVA											
Source	df	MSS	TSS	Titratable acidity	pH	Reducing Sugars	Viscosity				
		Total Solids									
Storage (S)	10	0.0005	0.003	0.0003**	0.025**	0.013	0.059**				
Drink type (D)	1	184.5**	148.6**	0.00005	0.00003	0.41**	0.135**				
S X D	10	0.001	0.0015	0.00003	0.0007	0.0014	0.005				
Error	44	0.772	0.035	0.00001	0.0052	0.033	0.013				

*significant at $p < 0.01$ ($n = 3$), ANOVA- Analysis of variance, df -degree of freedom, MSS- mean sum of squares

Table 5 Changes in colour (L, a, b) values of the cardamom flavoured sterilized milk drinks during storage

Type of drink	Storage(34.1±0.3 °C), days										
	0	15	30	45	60	75	90	105	120	135	150
L value											
Control	69.2	69.3	69.5	69.8	73.7	75.6	76.4	77.4	77.8	78.2	78.4
Low calorie	68.9	68.8	68.7	68.4	68.2	68.1	68.0	67.4	66.7	66.3	65.8
A value											
Control	1.6	1.7	2.0	2.2	2.4	2.7	2.7	3.0	3.0	3.2	3.4
Low calorie	2.2	2.4	2.7	3.1	3.4	3.6	3.9	4.1	4.1	4.3	4.5
B value											
Control	12.7	12.3	12.2	11.8	11.4	11.3	10.6	10.2	9.9	9.8	9.6
Low calorie	14.0	13.5	13.1	12.7	12.4	12.1	11.7	11.3	10.8	10.5	10.2
ANOVA											
Source	df	MSS									
		L	A	B							
Storage (S)	10	14.2**	35.7**	8.1**							
Drink type (D)	1	668.5**	15.2**	13.6**							
S X D	10	35.8**	0.06**	0.06*							
Error	44	35.7	0.004	0.027							

*significant at $p < 0.05$, ** significant at $p < 0.01$ ($n=3$), ANOVA- Analysis of variance, *df*-degree of freedom, *MSS*- mean sum of squares

browning during storage which led to development of slight burnt/bitter-sweet aftertaste in the drinks perceived by some of the sensory panelists. A similar trend in body and mouthfeel scores was observed with storage in both the drinks. This was due to reduction in viscosity and sedimentation of some proteins (Cano-Ruiz and Richter 1998). The overall acceptability scores were reduced significantly ($p < 0.01$) with storage. The score was higher for control than low calorie drink. Though the scores reduced considerably at the end of 150 days, both the drinks were highly acceptable. As reported by Gothwal and Bhavadasan (1992a), during 5 months storage of in-bottle sterilized milk samples, changes were not appreciable to cause defect of commercial importance. The shelf life of sterilized milk is variable and less clearly defined, as no easily detectable bacterial growth occurs. The deterioration in taste is affected by storage temperature, being 3 to 6 months in ambient temperature in moderate climates and by the presence of heat resistant lytic bacterial enzymes (Djmek 2011).

Changes in microbial counts of sterilized milk drinks during storage Standard plate count (SPC) was not detected up to 120 days of storage in control drink, thereafter a count (SPC) of $1.57 \log_{10}$ cfu/ml was observed which marginally increased to $1.70 \log_{10}$ cfu/ml at the end of 150 days. However, in low calorie drinks, standard plate count was detected only after 150 days of storage ($1.52 \log_{10}$ cfu/ml).

Added sucrose might have been the source of microbial spores in control drink. Most spores are thermophilic and thus survived the sterilization process to some extent and might have germinated during storage. Similar results were obtained for yeast and mold count (YMC) in both the drinks. YMC was not detected in low calorie drink up to 150 days of storage as compared to control where a count of $1.60 \log_{10}$ cfu/ml was observed. Likewise, spore formers were also detected earlier at 135 ($1.49 \log_{10}$ cfu/ml) days storage in control than that in low calorie drink at 150 days ($1.48 \log_{10}$ cfu/ml) of storage.

Conclusion

Low calorie drink containing sucralose and inulin was subjected to two thermal treatments i.e. pasteurization and sterilization and stored at refrigeration and room temperatures, respectively. The rate of changes in terms of TS, TSS, RS, viscosity, growth of SPC and yeast and mold was faster in former than latter during storage which directly affected the sensory attributes. A significant reduction in the flavour and body and mouthfeel scores was noticed in pasteurized drinks indicating the unacceptability of the drinks after 10 days of refrigerated storage. The shelf life of sterilized CFDs was estimated to be more than 5 months/150 days at room temperature (34.1 ± 0.3 °C).

Table 6 Changes in sensory scores of cardamom flavoured sterilized milk drinks during storage

Type of drink	Storage(34.1±0.3 °C), days										
	0	15	30	45	60	75	90	105	120	135	150
Appearance											
Control	8.5	8.5	8.3	8.3	8.3	8.2	8.2	8.1	8.1	8.1	8.1
Low calorie	8.3	8.2	8.2	8.2	8.1	8.1	8.0	7.9	7.8	7.8	7.8
Flavour											
Control	8.7	8.7	8.7	8.5	8.3	8.2	8.2	8.2	8.1	8.1	8.0
Low calorie	8.4	8.4	8.4	8.1	8.1	8.1	8.0	7.9	7.9	7.8	7.7
Body and mouthfeel											
Control	8.5	8.4	8.4	8.3	8.3	8.3	8.2	8.2	8.1	8.0	8.0
Low calorie	8.4	8.2	8.1	8.0	8.0	8.0	7.9	7.9	7.9	7.9	7.8
Overall acceptability											
Control	8.6	8.5	8.5	8.4	8.3	8.3	8.2	8.2	8.1	8.1	8.0
Low calorie	8.4	8.3	8.2	8.1	8.1	8.0	8.0	7.9	7.9	7.8	7.8
ANOVA											
Source	df	MSS	Appearance	Flavour	Body and mouthfeel	Overall acceptability					
Storage (S)	10	0.37		1.15**	0.284**	0.362**					
Drink type (D)	1	2.42**		4.51**	2.12**	2.93**					
S X D	10	0.296		0.084	0.148	0.090					
Error	154	0.220		0.237	0.103	0.102					

** significant at $p < 0.01$ ($n=8$), ANOVA- Analysis of variance, df -degree of freedom, MSS- mean sum of squares

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