Effect of modified atmospheric packaging on chemical and microbial changes in dietetic *rabri* during storage

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Abstract Rabri is a dairy based sweet popular in the Indian subcontinent. The high sugar and fat content impose restrictions on its consumption due to health reasons. Dietetic rabri was prepared by the replacement of sugar with aspartame. Inulin was added to partially replace the milk fat and to improve the consistency of rabri. The rabri samples were packed in the polyethylene bags filled with different gaseous compositions (Air, 50 % CO₂:50 % N₂ and 100 % N₂) and stored at 10 °C. The shelf life was evaluated on the basis of changes in the chemical quality parameters such as HMF, TBA and FFA and microbial content such as total plate count, yeast and molds and coliform counts. The chemical parameters and microbial spoilage increased in all the samples with the progression of storage period. The samples packed with air showed significantly higher chemical deterioration and microbial spoilage as compared to the other two combinations. The samples packed with 100 % N₂ were more shelf stable than with air and 50 % CO₂:50 % N₂ combinations.

Keywords Dietetic rabri · Modified atmospheric packaging · Inulin · Aspartame · Coliform

Introduction

India is the largest milk producing country in the world with an estimated **annual** production of 121.8 million tonnes (NDDB 2012). The major portion of milk produced (about 50 %) is

converted into traditional dairy products like heat **desiccated** milk products viz., *khoa*, *basundi*, *rabri* etc., coagulated milk products viz., *dahi*, *shrikhand*, *paneer*, *chhana* and *chhana* based products and clarified products viz. butter oil, *ghee*, etc. which are inherent in ancient traditions and have a strong social and cultural heritage in the Indian society.

Most of these traditional dairy products contain high levels of sugar and fat. The high sugar and fat content in dairy sweets impose restrictions on their consumption. According to International Diabetes Federation, diabetes currently affects 366.0 million people worldwide. India has the largest number of people with diabetes which is about 62.4 million (Mohan and Anbalagan 2013). Increase in the incidence of diabetes mellitus is mainly due to the modern life style and changing dietary patterns with balance tilted towards fabricated foods rich in sugar and fats. High consumption of sweetened products contributes to calories and obesity resulting in health problems. The discovery of a large number of sweeteners during the last decade has triggered the development of new sugar free products, particularly for diabetics, people on special diets and/or for the obese. Several artificial sweeteners incorporated low calorie products like low calorie flavoured milk (Arora et al. 2001, 2008; Bhardwaj et al. 2009), *burfi* (Arora et al. 2010), *kalakand* (Arora et al. 2008), *lassi* (George et al. 2012), dietetic *chhana kheer* (Gautam et al. 2012) and *lal peda* (Jain et al. 2012) have been reported.

Milk sweets during storage undergo several physical, biochemical and microbiological changes making them unfit for human consumption (Londhe et al. 2012). Consumers demand foodstuffs with superior quality and nutritional value as well as minimally processed foods retaining the fresh products' features. The demand for nutritious, functional and fresh food products have increased owing to changing life-style, economic growth and food preferences. This has brought a significant increase in the use of non-thermal packaging of food products including modified atmospheric

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packaging (MAP). Food products undergo changes during storage, showing adverse effects on quality, ranging from minor sensory defects to complete spoilage (Kotsianis et al. 2002). Several studies have been reported in the past resulting in an increase in the shelf life of traditional dairy foods using hurdle technology, water activity changes, increase in sugar content etc. (Kumar and Srinivasan 1982; Biradar et al. 1985; Thakur et al. 1992; Kumar et al. 1997; Sharma et al. 2001). However, not much of the scientific literature is available on use of MAP for extending the shelf life of traditional dairy foods except those reported by Londhe et al. (2012) for brown *peda* and Rai et al. (2008) and Thippeswamy et al. (2011) for *paneer*.

MAP has been used to preserve the freshness of many food products and can improve the food safety under certain conditions (Hotchkiss 1989; Farber et al. 1990). Compared to traditional product packaging methods, MAP offers many key benefits - the most important of which is extending the shelf life (Farber 1991). MAP of foods has proved to be capable of extending the shelf life of many foods by altering the relative proportions of the surrounding atmospheric gases. The gases normally used for MAP include CO₂, O₂ and N₂. Generally O₂ concentration must be below atmospheric levels (i. e. < 21 % v/v) (Farber 1991; O' Conner et al. 1992). CO₂ is primarily used as antimicrobial gas, which effectively inhibits the growth of spoilage bacteria and molds (Hotchkiss 1989). MAP can bring about changes in the respiration rate, microbial growth, oxidation reactions and thus impacts the shelf life of food products (Mangarj and Goswami 2009).

Rabri is a concentrated and sweetened whole milk delicacy containing several layers of clotted cream which is skimmed off from slowly evaporating milk. It is made from buffalo milk owing to its high total solid content which ultimately results in a final product with superior quality. The fat and sugar contents of *rabri* are 20 % each (dry weight basis) (Aneja et al. 2002), which may impose the restriction in its consumption for health conscious people. Also, the shelf life of traditional *rabri* is poor due to high moisture content.

A process for manufacturing dietetic *rabri* was optimized in our laboratory by replacing the sugar with aspartame and fat was reduced by using inulin as the fat replacer. The objective of this study was to determine the shelf life of dietetic *rabri* by packaging it under two combinations of nitrogen and carbon dioxide (50 % CO₂:50 % N₂ and 100 % N₂). The shelf life of *rabri* packed under MAP conditions was also compared with the conventionally packed *rabri* samples.

Materials and methods

Packaging material and condition s Polyethylene bags made from polyamide (20 µm), polyethylene (70 µm) with ethylene vinyl alcohol (EVC) was used for the packaging of dietetic

rabri samples. MAP was done using sealant layer oxygen, carbon dioxide and water vapour transmission rate for the pouches were 1,100 cm³/m².d, 2,700 cm³/m².d and 10 m².d, respectively under MAP conditions (nitrogen flushing in gas mixer Model: MAP Mix 9001 MK, Make: PBI Densensor, Ringsted, Denmark) packaged under vacuum in VAC-STAR* S220 MP, Make: VAC STAR, Sugiez, (Switzerland).

Preparation of dietetic rabri Dietetic *rabri* was prepared by the procedure standardized by De (1980) and by Gayen and Pal (1991) with slight modifications. Milk fat, aspartame and inulin levels were optimized in dietetic *rabri* by response surface methodology (RSM). Aspartame was used as a sugar replacer, while inulin was used as partial fat replacer as well as bulking agent. Aspartame and inulin were procured from Nutrasweet Co. (USA) and Cosucra Group (Belgium), respectively. The process was optimized for desired quality based on the sensory (colour and appearance, flavour, sweetness, body and texture and overall acceptability scores) and textural characteristics (cohesiveness, consistency, firmness and index of viscosity). For preparation of dietetic *rabri*, fresh raw milk was procured from the Dairy Farm, Banaras Hindu University, India and its fat and Solid Not Fat (SNF) contents were standardized to 4 % and 8.5 %, respectively. After straining, 10 kg standardized milk was taken in a shallow iron pan. Inulin (0.5 % of milk i.e. 50 g) was added to milk heated at simmering temperature (85–90 °C) and held undisturbed at this temperature by controlled heating. Milk was neither stirred nor allowed to boil. The surface of the milk was fanned to help the process of skin formation and the skin was collected separately. As soon as the volume of milk got reduced to one fifth of its original volume, the layer of skin collected was immersed in the mixture and allowed to cool at 37±5 °C. Then aspartame (0.16 % of milk i.e. 32 g) was added to the *rabri* with proper mixing. The final product (2.13 kg) was stored at 10±1 °C. Detailed process flow diagram for the manufacture of dietetic *rabri* is shown in Fig. 1. The composition of dietetic *rabri* was 38.7 % moisture, 25.1 % lactose, 20.8 % protein, 12 % fat and 2.9 % ash, the calorific value of the dietetic *rabri* was 291.6 kcal/100 g as compared to the traditional *rabri*, which contained 30 % moisture, 17 % lactose, 10 % protein, 20 % fat, 3 % ash and 20 % sugar and 368 kcal/100 g (De 1980).

Storage and analysis of fresh dietetic rabri Samples were packed in polyethylene bags under MAP conditions. Air was kept as control; the other combinations of gases were 50 % CO₂: 50 % N₂ and 100 % N₂. Samples were stored at 10 °C and withdrawn from storage at every 3rd day and analyzed for chemical and microbial properties. During the storage period, the development of hydroxyl methyl furfural (HMF) according to the method used by Keeney and Bassette (1969), Thiobarbituric acid (TBA) content using the method of Strange et al. (1977) and free fatty acids (FFA) according to

Fig. 1 Process flow diagram for the manufacture of dietetic *rabri* along with mass balance

the method given by Deeth et al. (1975) were measured. Microbial analysis viz. total plate count (TPC), yeasts and molds, and coliform counts was performed for all the samples by using standard plate count methods as described by Abdalla and Ahmed (2010). The moisture content in dietetic *rabri* samples was analyzed using the AOAC (2000) method.

Statistical analysis All the data were expressed as mean $\pm$ standard error of mean and calculated from three independent experiments. One-way analysis of variance (ANOVA) was applied and LSD was performed by using the Systat software to measure the test for significance by LSD post hoc test as described by Snedecor and Cochran (1989).

Results and discussion

Moisture content Among samples A (air filled), B (50 % CO₂+50 % N₂) and C (100 N₂), the moisture content varied significantly ($p < 0.05$). It can be seen from Fig. 2a that there was a gradual loss of moisture in all the samples throughout the storage period of 30 days. However, the moisture loss was the highest in the control sample packed under air, whereas, the samples stored in MAP conditions showed comparatively less loss of moisture. Among MAP packed samples, those stored in 100 % N₂ showed highest retention of moisture and the least deviation in moisture content among replicates. Therefore it can be stated that the MAP of dietetic *rabri* stored with 100 % N₂ could serve better for retention of moisture.

Hydroxy methyl furfural (HMF) Maillard reaction is the most common browning reaction which occurs during thermal

Fig. 2 Chemical changes in dietetic *rabri* during storage at 10 °C under different atmospheres; **a**) moisture loss (%) **b**) HMF content (μ moles/100 g) **c**) TBA (Absorbance at 532 nm) **d**) FFA (μEq/g) Each value is a mean $\pm$ standard error mean ($n=3$)

processing and storage of milk and milk products (Palton 1952). This reaction is often characterized by the production of HMF; an intermediate product of Maillard reaction. The HMF content of the MAP packaged *rabri* samples, stored at 10 °C and packaged with varying gas percentage viz. air filled (A), 50 % CO₂:50 % N₂ (B) filled and 100 % N₂ (C) filled was estimated. The average HMF content increased from 3.02 $\pm$ 0.06 to 3.37 $\pm$ 0.05 μ moles/100 g and 3.02 $\pm$ 0.04 to 3.33 $\pm$

0.05 μ moles/100 g for sample B and C, respectively (Fig. 2b). The control sample (A) exhibited the highest HMF content after 9 days (3.38 ± 0.04 μ moles/100 g) which indicated that the control sample was more prone to Maillard reaction than the samples, B and C. There was a significant difference in HMF content among sample A, B and C at $p < 0.05$. Londhe et al. (2012) studied the effect of packaging techniques on the shelf life of brown *peda*, a milk-based confection. They reported that during the storage, highest increase in HMF content was observed in control samples packaged in cardboard boxes on the 20th day of the storage and lower value in MAP packaged (40 % CO₂:55 % N₂ and 60 % CO₂:40 % N₂) samples, which was in agreement with the current findings.

Thiobarbituric acid (TBA) TBA levels indicate the oxidative changes in foods especially in fatty foods like *rabri*, where it normally increases with storage time. In the present study, the TBA value for all the samples showed an increasing trend during the storage (Fig. 2c). The average TBA values of the sample A at 10 °C, changed from an initial 0.08 ± 0.01 to 0.22 ± 0.03 on the 9th day of the storage. TBA values of samples B and C, increased from 0.08 ± 0.01 to 0.23 ± 0.03 and 0.08 ± 0.01 to 0.25 ± 0.03 , respectively on 9th day of storage. There was a significant difference in TBA values among sample A, B and C at $p < 0.05$. Meshref and Al-Rowaily (2008) reported an increase in TBA value in milk and other dairy products due to heating. The high TBA values exhibited in dietetic *rabri* could be attributed to oxidative changes during storage.

Free fatty acid (FFA) The microorganisms and enzymatic activities which lead to lipid hydrolysis are eliminated during preparation of dietetic *rabri*. The FFA content for the sample A increased from 1.24 ± 0.14 to 28.2 ± 2.90 μ eq/g after 9th day of the storage. The FFA value increased from 1.24 ± 0.19 to 36.7 ± 2.56 μ eq/g and 1.24 ± 0.12 to 29.31 ± 2.15 μ eq/g for samples B and C, respectively (Fig. 2d) from day 0 to day 30. There was a

significant difference in FFA content among samples A, B and C at $p < 0.05$. FFA is an indicator of deterioration of lipid component in food product and is reported to increase during heating of the milk products (Meshref and Al-Rowaily 2008). Thus, thermal processing of *rabri* possibly leads to high initial content of FFA. The sample A exhibited higher FFA content of 28.2 ± 2.90 μ eq/g in 9 days than those of the MAP samples (B and C) which means that MAP treatment could prevent the samples from getting rancid. The samples packed with 100 % N₂ possessed the lowest FFA value making it least rancid in the sample set. Londhe et al. (2012) studied the effect of packaging techniques on the shelf life of brown *peda*, a milk-based confection. They reported that during storage, highest increase in free fatty acid (FFA) was observed in control samples packaged in cardboard boxes on the 20th day of storage as compared to the MAP packaged samples. Current findings are in accordance with their observations.

Microbial quality of MAP packaged dietetic rabri Microbial analysis of dietetic *rabri* was performed to evaluate the microbial quality of the product during storage. The results obtained are presented in Table 1. The bacterial and 'yeast and mold count' of the Sample A, B and C varied significantly ($p < 0.05$) after 3 days of storage. The sample A showed maximum microbial count of $6.4 \log_{10}$ CFU/g of the sample after 9 days of storage while, Sample B and C had lower microbial count. Also, the microbial count of sample B and C varied significantly ($p < 0.05$) in comparison with each other after 3 days of storage. Test for coliform was performed for all the samples and no colonies appeared in any sample. Smith et al. (1986) reported that in the gas packaged (40 % N₂:60 % CO₂) crusty rolls with the headspace O₂ concentration never increased beyond 0.05 % and the rolls remained mold-free even after 60 days. A similar mold-free shelf-life was obtained in air or N₂ packaged crusty rolls, which was comparable with our current findings.

Table 1 Changes in microbial counts ($\log_{10}$ CFU/g) of dietetic *rabri* during storage at 10 °C under different atmospheres

	No of days	Total plate count			Yeast and molds		
		Air	50%CO ₂ :50%N ₂	100%N ₂	Air	50%CO ₂ :50%N ₂	100%N ₂
	0	5.4 ± 0.6^a	5.2 ± 0.6^a	5.2 ± 0.6^a	5.6 ± 0.5^a	5.6 ± 1.1^a	5.6 ± 1.0^a
	3	5.9 ± 1.1^a	5.4 ± 0.6^a	5.2 ± 1.1^a	6.0 ± 0.8^a	5.7 ± 1.1^a	5.6 ± 1.0^a
	6	6.2 ± 0.5^a	5.9 ± 1.0^b	5.3 ± 0.7^c	6.2 ± 0.6^a	6.0 ± 0.7^a	5.7 ± 1.0^b
	9	6.4 ± 1.0^a	6.0 ± 1.2^b	5.4 ± 0.5^c	6.4 ± 0.7^a	6.2 ± 0.5^b	5.7 ± 0.2^c
	12	—	6.1 ± 0.5^a	5.6 ± 0.3^b	—	6.2 ± 1.0^b	5.7 ± 0.7^b
	15	—	6.1 ± 1.0^a	5.7 ± 1.1^b	—	6.2 ± 0.5^a	5.8 ± 0.5^b
	18	—	6.1 ± 1.0^a	5.8 ± 0.6^b	—	6.2 ± 0.8^a	5.8 ± 1.0^b
	21	—	6.1 ± 1.0^a	5.8 ± 1.2^b	—	6.3 ± 0.2^a	5.8 ± 0.8^b
	24	—	6.1 ± 1.0^a	5.8 ± 0.6^b	—	6.3 ± 0.6^a	5.9 ± 0.6^b
	27	—	6.2 ± 0.5^a	5.9 ± 1.1^b	—	6.3 ± 1.1^a	5.9 ± 1.0^b
	30	—	6.2 ± 1.0^a	5.9 ± 0.4^b	—	6.3 ± 0.5^a	5.9 ± 0.6^b

Values are mean $\pm$ standard deviation ($n=3$)

Means in the rows with different superscripts are significantly different ($p < 0.05$)

CFU Colony forming units

Conclusion

The data obtained from chemical and microbial analysis of dietetic *rabri* packaged with MAP filled with different combinations of gases i.e. air, N₂ and CO₂ and stored at 10 °C showed that the dietetic *rabri* is shelf stable for 30 days at 10 °C at both the gas combinations of MAP. Though, the control samples spoiled after eight days, 50 % CO₂:50 % N₂ and 100 % N₂ samples remained acceptable during the storage study of 30 days. The control sample had considerably higher HMF, TBA, FFA and also microbial load which indicates that MAP had a positive effect on the storage of the samples, especially 100 % N₂ packed was the best as compared to air and 50 % CO₂:50 % N₂. This study has proven that MAP can improve the shelf life of the *rabri* to an appreciable extent thus giving a promising indication on enhancing its marketability and consumerism.

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