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Chemical changes in unbranded and branded indigenous fermented dairy products during refrigerated storage

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Abstract

Indigenous fermented dairy products such as misti doi, shrikhand, lassi and butter milk collected from Unbranded and Branded sources revealed increase in the Titratable Acidity (TA) and decrease in Free Fatty Acid (FFA) and in protein content. Mean rise in TA and FFA ranged from 0.50 - 0.79% LA and 0.11 - 28% FFA in branded untreated mishti doi samples whereas branded UHT treated samples had 1.85-2.64% LA and 0.45-0.82%, protein content reduced from 3.61-0.28 and 1.99-1.12% in untreated and UHT treated samples respectively. Acidity and FFA of Unbranded shrikhand samples was in range of 0.45-0.75% LA and 2.18-3.27% while of branded samples was 0.26-1.13 and 1.29- 3.75% respectively. Reduction in protein content was from 1.49 to 0.86 (unbranded); 1.92 to 0.12 (branded shrikhand). Unbranded lassi samples showed rise in acidity from 0.19-0.36% LA and 3.12- 6.00% FFA, where as branded samples revealed 0.15-0.46% LA and 1.89-3.29% FFA. Unbranded lassi samples showed reduction in protein from 1.72-1.04 whereas branded lassi samples revealed 1.95-0.66%. Increase in acidity and FFA from 0.17-0.45% LA and 2.16-3.52% was observed in unbranded buttermilk samples while branded samples revealed 0.24-0.57% LA and 1.30-3.70%. Protein content of unbranded butter milk samples reduced from 2.68 to 1.45 where as that of branded samples from 1.12 to 0.16%. Statistical analysis of data revealed that mishti doi and lassi samples showed significant increase in titratable acidity, shrikhand samples revealed significant rise in both TA and FFA content but butter milk samples revealed significant decrease in protein content.

Keywords: Unbranded, branded, refrigerated storage, chemical deterioration, acidity and psychrotrophs

Introduction

Indigenous dairy products refer exclusively to milk products of a particular region of India. India with many states has varieties of milk products, that include those prepared by the deliberate addition of starter organisms termed as Indigenous Fermented Dairy Products (IFDPs). Fermentation leads to lowering the pH, increasing the shelf life of the product. The refrigeration storage can postpone the spoilage as it prevents mesophilic microorganisms but not the psychrotrophs. The acidic pH and refrigerated storage of indigenous fermented dairy products become selective for psychrotrophic molds that results in spoilage of these refrigerated products by acting upon milk components and releasing end products such as lactic acids, amino acids and free fatty acids. These end products induce spoilage of these indigenous fermented milk products through off flavours: fermented, yeasty or musty and gassy appearance (Filtenberg *et al.*, 1996) [2].

Flavour defects such as fruity, alcoholic, highly acidic taste were observed in the market samples of Mishti doi (Ghosh and Rajorhia, 1990) [4]. Release of short chain fatty acids, C_{4:0} through C_{8:0} resulted in rancid flavours and odours, whereas the release of long chain fatty acids resulted in a soapy flavour which was considered as off flavour defect in milk (Sorhaug and Stepaniak, 1991; Shah, 1994) [13, 10].

Various brands of IFDPs are available in the market as well few products are sold in bakeries and sweet meat shops without any branding. The studies regarding chemical changes in refrigeration stored indigenous fermented dairy products are very limited. With this background many unbranded and branded samples of indigenous fermented dairy products such as mishit doi, shrikhand, lassi and buttermilk samples from different areas of Bengaluru city were selected and studied for major chemical changes till their spoilage.

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Materials and Methods

1. Collection of Indigenous Fermented Milk Product Samples

Various unbranded and branded Indigenous Fermented Dairy Products such as Mishti doi, Shrikhand, Lassi and Buttermilk were collected from retail outlets and local sweetmeat shops from Hebbal, Banashankari, R.T. Nagar and Majestic areas of Bengaluru city for chemical analysis and stored under refrigeration conditions till analysis (7 °C).

2. Chemical Analysis of Indigenous Fermented Dairy Products

- a) **Determination of titra table acidity, protein and free fatty acid:** The samples collected were analyzed for acidity (%LA), protein and Free Fatty Acid (FFA) to study the effect of refrigerated storage on the quality of the market samples of Indigenous Fermented Dairy Products. The titratable acidity and free fatty acid content of the samples were determined by standard methods followed by BIS (SP: 18(Part XI)-1981), while Pyne's method (Hill and Stone, 1964) was adapted for estimation of protein content in samples
- b) **Statistical analysis:** The data obtained in present study was analyzed using one way ANOVA to calculate the mean and F-values and to study significant or non-significant effect of the parameters using R- software (R. version 3.1.3, 2015).

Results and Discussion

Mishti doi

Branded Mishti doi samples of both untreated and UHT treated (MDB1, MDB2, MDB3 and MDB4) were analyzed for acidity, percent protein and FFA during their refrigerated storage. The mean values among untreated samples MDB1, MDB2 and MDB3 for acidity ranged from 0.50 to 0.79 while for protein content from 3.61 to 0.28 and for FFA from 0.11 to 0.28 where as UHT treated sample MDB4 showed the mean values of 2.64, 1.12 and 0.82 for acidity, protein and FFA content respectively. The untreated samples were acceptable till 45days with higher FFA content while that of UHT treated samples spoiled after 60days with high acidity and lowest protein content than untreated samples that might be due to the prolonged storage period at 7 °C. Statistical analysis revealed significant ($p < 0.05$) rise in acidity among the samples (Table 1).

Ray and Srinivasan (1972) [8] reported increase in lactic acid content in mishit doi during refrigerated storage. The same results were obtained in mishit doi samples of Bengaluru market. In relevance with current study, syneresis (wheying off) was reported in mishit doi samples which was considered as spoilagesign reason being proteolysisby Salvador and Fiszman (2004) [9].

Shrikhand

Unbranded (SUB1, SUB2 and SUB3) and branded (SB1, SB2, SB3 and SB4) shrikhand samples of Bengaluru city were analyzed for chemical changes. The results revealed mean range for acidity, protein and FFA content from 0.43-0.75% LA, 1.49-0.86% and 2.18-3.27% in unbranded samples whereas in branded samples ranged from 0.256- 1.13% LA, 1.92-0.11% and 1.285-3.76% respectively. The branded samples due to prolonged storage might have revealed higher rise in acidity and FFA and reduction in protein content than unbranded samples. Thelactic acid and FFA increased

significantly as per the statistical analysis (Table 2A and B).

Protein content of the samples reduced as storage period increased which might be due proteolysis by the hydrolytic proteinases of molds. In relevance to the present results obtained, Sharma and Zarinwala (1980) [11] also observed rise in acidity and reduction in protein content during their storage (after 10days and 30days) in shrikhand at room and refrigerated temperature.

An increase in the FFA content was recorded during storage. The lowest FFA content was in SUB2 (2.48%) whereas SUB3 showed maximum of 5.37% FFA. In the branded samples SB1 had minimum (2.29%) than SB3 (3.75% FFA). This increase might be due to the activity of extracellular lipases of psychrotrophic molds which was found to be active even at low temperature that resulted in deterioration of flavour (Bhatt *et al.*, 2010) [1]. It was surprising to note that the quality of unbranded shrikhand was better with respect to protein content rather than acidity and FFA contents due to the shorter shelf life of 45days compared to branded samples having 120 days of shelf life. On par with the study, product attained spongy texture with gas production during its spoilage (Nigam *et al.*, 2009) [6].

Lassi

The unbranded (LUB1 and LUB2) and branded (LB1 and LB2) lassi samples collected from market of Bengaluru city were analysed for chemical changes. The mean increase in acidity in unbranded samples were from 0.19 to 0.35% LA and 0.15 to 0.46% in branded lassi samples. FFA content increased tremendously from 3.12 to 6.00% in unbranded samples while in branded samples was from 1.89 to 3.29% FFA in 20 days of refrigerated storage which is surely due to action of extracellular hydrolytic mold lipases. Highest rise in acidity and lowest protein content was observed in branded lassi samples might be due to prolonged storage which induced enzymatic degradation where as more rise of FFA was observed in unbranded samples due to the mold lipase added from unhygienic handling practices.

In an agreement to the present study, even George *et al.*, (2010) [3] also have concluded that rise in acidity might be due to mold growth that induced rancidity in samples. Activity of extra cellular psychrotrophic mold proteinase might have led to reduction in protein content to 1.04 from 1.72 per cent in unbranded samples while in branded samples it was 0.67 from 1.95 per cent. On par with the present study, Shuwu *et al.* (2011) [12] also observed reduction in fat and protein content during storage (7 °C) of lassi samples. The rise in LA content was significant during refrigerated storage (Table 3A and B).

Butter milk

Analysis of an Unbranded (BMUB1) and five branded (BMB1, BMB2, BMB3, BMB4 and BMB5) buttermilk samples of Bengaluru city indicated values for acidity, protein content and lipid content as 0.17, 2.68 and 2.16 initially and 0.45 per cent LA, 1.45 per cent protein and 3.52 per cent of FFA on 20th day for BMUB1. Unbranded buttermilk sample had initial acidity of 0.17 and raised to 0.45 where as protein content which was 2.68% came down to 1.45, while % FFA which was 2.16 increased to 3.52 at the end 20 days of storage. BMB1, BMB3, BMB4 spoiled after 90 days while BMB2 and BMB5 showed 150 days and 120 days of shelf life respectively. For branded samples mean value for acidity ranged from 0.24 to 0.67, protein ranged from 1.13 to 0.16, while FFA ranged from 1.30 to 3.70. Prolonged storage of

branded samples resulted in the highest rise in TA and FFA where as higher Proteinase activity resulted in highest reduction in protein content of unbranded samples. Statistically significance ($p < 0.05$) was noticed in reduction of protein content among the samples during storage period (Table 4A and B).

Swapna and Chavannavar (2012) [15] concluded that as storage

periodin refrigeration of buttermilk increased reduction in protein content was observed. Contradictory to the present study, Vasavada and White (1979) [16] observed off flavour development in butter milk samples from market stored at 7 °C in 7 days while better results with shelf life of 10 days was noticed in unbranded whereas 90 to 150 days in branded UHT butter milk samples.

Table 1: Major chemical changes in branded untreated and UHT treated mishtidoi samples during refrigerated storage (7 °C)

Storage period (Days)	Untreated												UHT treated		
	MDB1			MDB2			MDB3			Mean			MDB4		
	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA
00	0.17	3.61	0.09	0.95	3.12	0.16	0.39	4.10	0.09	0.50	3.61	0.11	1.85	1.99	0.45
10	0.35	3.03	0.15	1.32	2.70	0.34	0.57	3.45	0.23	0.75	3.06	0.24			
20	0.58	2.61	0.27	1.78	1.17	0.63	0.79	3.03	0.39	1.05	2.27	0.43			
30	Spoiled			2.36	0.84	0.85	Spoiled			0.79	0.28	0.28	2.18	1.61	0.60
40	Spoiled			Spoiled			Spoiled			Spoiled					
50	Spoiled			Spoiled			Spoiled			Spoiled					
60	Spoiled			Spoiled			Spoiled			Spoiled			2.64	1.12	0.82
70	Spoiled			Spoiled			Spoiled			Spoiled			Spoiled		

F-value(LA) = 11.1 ***, F-value(PRT) = 0.782, F-value(OA) = 0.04

Note: a) LA - Lactic Acid b) PRT - Protein content c) FFA - Free fatty acid content

Table 2: Major chemical changes in unbranded and branded shrikhand samples during refrigerated storage (7 °C)

A Unbranded Shrikhand

Storage period (Days)	SUB1			SUB2			SUB3			Mean		
	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA
0 days	0.81	1.83	2.25	0.33	1.88	1.27	0.15	0.76	3.01	0.43	1.49	2.18
15 days	0.86	1.61	3.04	0.40	1.50	1.62	0.25	0.50	3.79	0.50	1.20	2.82
30 days	0.91	1.28	3.48	0.49	1.26	2.22	0.41	0.27	5.37	0.60	0.94	3.69
45 days	0.97	0.90	4.05	0.53	0.82	2.48	Spoiled			0.75	0.86	3.27
60 days	Spoiled											

B Branded Shrikhand

Storage period (Days)	SB1			SB2			SB3			SB4			Mean		
	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA
0	0.11	3.27	0.85	0.28	3.10	1.12	0.40	0.76	1.41	0.32	0.56	1.76	0.26	1.92	1.29
30	0.12	2.46	1.05	0.40	2.38	1.13	0.53	0.59	2.20	0.48	0.21	2.12	0.36	1.41	1.63
60	0.12	1.29	1.45	0.57	1.88	1.78	0.71	0.41	2.72	0.63	0.09	2.53	0.51	0.92	2.12
90	0.21	1.11	2.29	0.71	1.55	2.67	0.96	0.12	3.04	Spoiled			0.63	0.93	2.67
120	Spoiled						1.13	0.11	3.75	Spoiled			1.13	0.11	3.75
150	Spoiled						Spoiled			Spoiled			Spoiled		

F- value (LA) = 0.755***, F- value(PRT) = 0.091, F- value(FFA) = 9.798***

Note: a) LA - Lactic Acid b) PRT - Protein content c) FFA - Free fatty acid content

Table 3: Major chemical changes in unbranded and branded lassi samples during refrigerated storage (7°C)

A Unbranded lassi

Storage period (Days)	LUB1			LUB2			Mean		
	%LA	%PRT	%FFA	%LA	%PRT	%FFA	%LA	%PRT	%FFA
0 days	0.32	0.60	1.94	0.11	2.84	4.3	0.19	1.72	3.12
10 days	0.48	0.38	2.48	0.16	2.30	6.96	0.29	1.34	4.72
20 days	0.55	0.30	3.09	0.25	1.78	8.92	0.35	1.04	6.01
30 days	Spoiled								

B Branded lassi

Storage period (Days)	LB1			LB2			Mean		
	%LA	%PRT	%FFA	%LA	%PRT	%FFA	%LA	%PRT	%FFA
0 days	0.08	2.68	2.4	0.23	1.21	1.38	0.16	1.95	1.89
30 days	0.11	1.88	2.79	0.37	0.73	1.73	0.24	1.30	2.26
60 days	0.25	1.45	3.01	0.42	0.41	2.39	0.34	0.93	2.70

90 days	0.40	1.10	3.58	0.52	0.22	2.99	0.46	0.66	3.28
120 days	SPOILED								
F- value (LA)= 0.040***, F- value (PRT)=0.111, F- value(OA)= 4.336									

Note: a) LA - Lactic Acid b) PRT - Protein content c) FFA - Free fatty acid content

Table 4: Major chemical changes in unbranded and branded buttermilk samples during refrigerated storage (7°C)
A Unbranded Buttermilk

Storage period (Days)	BMUB1		
	%LA	%PRT	%FFA
0 days	0.17	2.68	2.16
10 days	0.29	1.88	2.71
20 days	0.45	1.45	3.52
30 days	Spoiled		

B Branded butter milk

Storage Period (Days)	BMB1			BMB2			BMB3			BMB4			BMB5			Mean		
	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA	% LA	% PRT	% FFA
00	0.49	0.78	1.21	0.22	0.70	1.11	0.29	0.64	1.48	0.12	2.44	1.56	0.08	1.07	1.15	0.24	1.13	1.30
30	0.64	0.50	1.73	0.36	0.66	1.25	0.41	0.51	1.59	0.28	2.12	2.31	0.13	0.83	1.51	0.36	0.92	1.68
60	0.81	0.35	2.42	0.43	0.39	1.77	0.51	0.41	1.83	0.44	1.43	3.23	0.33	0.48	2.01	0.50	0.61	2.25
90	0.89	0.22	3.14	0.56	0.35	2.86	0.57	0.20	2.25	0.59	0.88	4.07	0.55	0.23	2.64	0.63	0.38	2.99
120	Spoiled			0.63	0.28	3.33	Spoiled						0.81	0.19	3.15	0.72	0.24	3.24
150				0.67	0.16	3.70							0.67	0.16	3.70			
180				Spoiled									Spoiled					

F- value (LA)=3.175, F- value (PRT)= 17.663*** and F- value (FFA)=3.977

Note: a) LA - Lactic Acid b) PRT - Protein content c) FFA - Free fatty acid content

Conclusion

Unbranded and branded Indigenous Fermented Dairy Products like Mishtidoi, Shrikhand, Lassi and Buttermilk collected from retail outlets and local sweetmeat shops from Hebbal, Banashankari, R.T. Nagar and Majestic areas of Bengaluru city were stored under refrigeration conditions (7 °C) and carried out chemical analysis. Among unbranded and branded Indigenous Fermented Dairy Products collected, unbranded ones showed lower reduction than branded samples which may be due to early consumption of unbranded (shelf life of 20 days) compared to branded (shelf life of 90 days) ones. Mishti doi and lassi samples showed significant increase in titratable acidity, shrikhand samples revealed significant rise in both TA and FFA content whereas butter milk samples revealed significant decrease only in protein content.

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