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Microbiological quality of Shrikhand during refrigerated storage ($5 \pm 2^{\circ}\text{C}$)

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Abstract

Shrikhand was prepared from the base material chakka and its storage study was done for refrigerated condition ($5 \pm 2^{\circ}\text{C}$) using two types of cultures *Lactococcus lactis* sub sp. *cremoris* R₆ (S₁) and *Lactobacillus bulgaricus* W + *Streptococcus thermophilus* H (S₅). It was observed that the standard plate count of shrikhand showed increasing trend upto 20 days for the samples stored at refrigerated temperature. Yeast and mould counts exhibited the same type of observation. No coliform was found in the shrikhand samples. The thermophilic, thermoduric and psychrotrophic counts of shrikhand prepared by S₁ and S₅ cultures separately, increase significantly as the storage days proceeds in the storage at refrigerated condition. Shrikhand stored at refrigerated condition showed significant increase in acidity upto 50 days but it was not organoleptically acceptable after 50 days of storage.

Keywords: Shrikhand, microbiological quality, group count profile and titratable acidity

Introduction

Since, milk is a highly perishable article of food, various methods of preserving it have been adopted. The practice of preserving milk by fermentation is a common household technology in India. Fermented milks are mild to strongly acidic in taste with pleasant aroma. Today, the practice of preserving milk by fermentation has become a facile household technology and is an important item of the diet in this sub-continent. Fermented milk products have been curative effect against gastro-intestinal disorders (Singh *et al.*, 1979; Renner, 1974) ^[9, 7] and have been utilized by many civilizations.

Shrikhand is an indigenous, concentrated, sweetened and lactic fermented milk product popularly and widely consumed as sweet dish in Maharashtra, Gujarat and part of Karnataka States. Chakka, a base material for shrikhand is prepared from dahi by isolating a major portion of milk-solids through staining and hanging the curd for draining of whey. Chakka may be kneaded with sugar and colour and flavouring materials may be added to it to get a well-accepted shrikhand for the consumers. The solid mass chakka contains an average of 63.2 percent moisture, 14.7 percent fat and 0.8 percent lactic acid (De, 1980) ^[6].

Shrikhand is also being manufactured on an industrial scale. Incorporation of modern technology and radical innovations in the traditional practices have made possible, the manufacture of safe, uniform, high quality product on an industrial scale and expand the market segment and improve its financial performance. As a major enterprise, the market for fermented milk products industry is growing in India. As per market potential is considered National Dairy Development Board (NDDB) has taken all out efforts to improve upon the marketability of milk and milk products.

Shrikhand has become popular among various sections of people due to its taste, energy content, higher nutritive value (Atreja and Deodhar, 1987; Choudhary and Atreja, 1985) ^[2, 5], flavour etc.

The present study has been undertaken with a view to obtain better quality chakka and shrikhand prepared in laboratory and comparison of shelf life of these products by selection of suitable strains of lactic acid bacteria for the preparation of shrikhand. The best starter culture has been selected on the basis of lower setting time of curd and higher acidity produced with different lactic acid bacteria either singly or in combination. The final product was analysed chemically and microbiologically to study the shelf life of the products to increase their acceptability by the consumers and the dairy industry.

Materials and Methods

a) Type of milk

1. Autoclaved reconstituted skim milk (0.1% fat, 9% SNF) was used for stater propagation and to determine stater activity.
2. Fresh whole raw milk (4.5% fat and 8.5% SNF) was collected from Students Dairy, situated near the Faculty of Dairy Technology, W.B.U.A.F.S., Mohanpur, Nadia, W. B.

b) Sources of starter cultures

Ampoules of four dried cultures of (i) *Lactobacillus bulgaricus* W (ii) *Streptococcus thermophilus* H (iii) *Lactococcus lactis* sub sp. *lactis* 287 and (iv) *Lactococcus lactis* sub sp. *cremoris* R₆ were obtained from National collection of Dairy Cultures, National Dairy Research Institute, Karnal, India.

All the cultures were activated and maintained in the autoclaved reconstituted skim milk fortified with 1% dextrose, 0.1% yeast extract. Stater cultures activated at 30 ± 2°C (*Lactococcus lactis* sub sp. *lactis* 287 and *Lactococcus lactis* sub sp. *cremoris* R₆) and 40 ± 2°C (*Lactobacillus bulgaricus* W and *Streptococcus thermophilus* H) by three successive transfers at 24 hr intervals before use.

c) Sources of sugar

Fine crystalline cane sugar was purchased from the local market situated in Kancharapara, W.B.

d) Sources of skim milk powder

Skim milk powder commercial 'SAGAR' brand was purchased from local market.

e) Selection of starter cultures

Five different combinations of starter cultures were used for the preparation of shrikhand. From among the different starter cultures, used for the preliminary trials in the preparation of dahi, intended for shrikhand manufacture. Finally a two starter culture *Lactococcus lactis* sub sp. *cremoris* R₆ (S₁) and combination of *Lactobacillus bulgaricus* W and *Streptococcus thermophilus* H (S₅) were selected for the further use in the preparation of dahi on the basis of their superiority with the rate of acid development as well as good organoleptic tests.

f) Flow Diagram of Preparation of Shrikhand

Dahi (acidity 0.7-0.8% LA) → Drainage of whey (hanging in muslin cloth for 12 hr) → Chakka → Addition of sugar @ 40% of chakka → Kneading → Uniform mixing → Addition of flavour (cardamom @ 1%) → Shrikhand → Filling → Storage (below 5°C)

g) Analytical methods

For the estimation of various microbial populations in the samples of shrikhand stored at 5 ± 2°C, the microbiological examinations mentioned below along with testing of titratable acidity have been carried out till the quality of samples remained good in terms of:

1. Standard Plate Count [BIS (1960) IS : 1479]
2. Coliform Count [BIS (1973) IS : 7035]
3. Yeast and mold Count [BIS (1973) IS : 7035]
4. Titratable acidity [BIS (1960) IS : 1479]

5. Thermophilic Count [APHA (1978)]
6. Thermoduric Count [APHA (1978)]
7. Psychrotrophic Count [APHA (1978)]

Results and Discussion

a) Microbiological quality of Shrikhand during refrigerated storage (5 ± 2°C)

It is revealed from the Table-1, that the average microbiological quality of shrikhand prepared by using *Lactococcus lactis* sub sp. *cremoris* R₆ (S₁) were found to be standard plate count, coliform count and yeast and mould count as 1.496 -11.37 × 10⁶/g, 0.0-0.0 × 10/g and 1.506-56.6 × 10/g respectively. Similarly, the counts were obtained for shrikhand samples prepared by using *Lactobacillus bulgaricus* W + *Streptococcus thermophilus* H (S₅) culture as 5.66-45.26 × 10⁶ spc/g, 0.0-0.0 × 10 coliform count/g and 1.816-69.13 × 10 yeast and mould count/g. Both the results, for shrikhand samples were not similar to the results reported by Sharma and Zariwala (1980) [8]. The standard plate count showed a significant increase in counts. No coliform count found in shrikhand samples. Upadhyay *et al.* (1975) [10] reported that there is wide variation in the viable count of market samples of shrikhand (24,500 to 16,50,000/g) due to variation in the level of contamination, types of contaminations, acidic pH of the product, sugar concentration and storage temperature and time. An increase in yeast and mould count of shrikhand samples upto 20 days was observed. Results of the present investigation in the count of yeast and mould were in conformity to those reported by Upadhyay *et al.* (1975) [10].

b) Group count profile study of Shrikhand during refrigerated storage (5 ± 2°C)

From the Table-2, it was evident that the group count profile study of shrikhand samples increased progressively. Shrikhand samples prepared from *Lactococcus lactis* sub sp. *cremoris* R₆ (S₁) showed thermophilic, thermoduric and psychrotrophic counts in the range of 101.3-713.6 × 10³ /g, 97.0-685.7 × 10³/g and 12.0-83.82 × 10³/g respectively. Similarly, the counts were obtained for shrikhand samples prepared by using *Lactobacillus bulgaricus* W + *Streptococcus thermophilus* H (S₅) culture showed in the range of 476.6-3351.6 × 10³/g, 427.0-2991.6 × 10³/g and 9.83-68.86 × 10³/g respectively. It is observed that there is significant increase in group counts during refrigerated storage. The results obtained for the shrikhand samples varies significantly between the shrikhand samples prepared by using two different cultures, which may be due to the effect of cultures on the different groups under study.

c) Development of Acidity for Chakka and Shrikhand in terms of % lactic acid during refrigerated storage (5 ± 2°C)

The development of acid in terms of lactic acid % is expressed in Table-3 for chakka and shrikhand stored in refrigerated condition. The chakka samples were stored upto 20 days for samples at refrigeration condition. The chakka samples showed significant increase of acidity at refrigerated storage upto 20 days after which they were refrigerated organoleptically. From Table-3, it is observed that shrikhand prepared from both the cultures showed significant increase of acidity upto 50 days but they were not organoleptically accepted at 50 days of storage.

Table 1: Microbiological quality of Shrikhand during refrigerated storage (5 ±2⁰C)

Type of culture used	Days of storage	SPC × 10 ⁶ /g	Coliform count × 10/g	Yeast and Mould count × 10/g
S ₁	0	1.496	Nil	1.506
	10	2.908	Nil	4.76
	15	9.423	Nil	34.2
	20	11.371	Nil	56.6
S ₅	0	5.66	Nil	1.186
	10	11.33	Nil	6.23
	15	38.16	Nil	41.30
	20	45.26	Nil	69.133
Combined Mean (S ₁ + S ₅)		15.70	0	26.91
SEm ±		3.83	0	1.45
C. D.		8.45	0	3.21

* p < 0.05

S₁ – *Lactococcus lactis* sub sp. *cremoris* R₆.

S₅ – *Lactobacillus bulgaricus* W + *Streptococcus thermophilus* H.

Table 2: Group count profile study of Shrikhand during refrigerated storage (5 ±2⁰C)

Type of culture used	Days of storage	Thermophilic count × 10 ³ /g	Thermoduric count × 10 ³ /g	Psychrotrophic count × 10 ³ /g
S ₁	0	101.3	97.0	12
	10	183.2	176.1	21.53
	15	519.1	568.1	69.51
	20	713.6	685.7	83.82
S ₅	0	476.6	427	9.8
	10	861.3	770.1	17.3
	15	2782.01	2480.2	57.953
	20	3351.6	2991.6	68.86
Combined Mean (S ₁ + S ₅)		1123.6	1024.48	42.607
SEm ±		294.5	253.38	1.578
C. D.		648.2	557.70	3.474

* p < 0.05

S₁ – *Lactococcus lactis* sub sp. *cremoris* R₆.

S₅ – *Lactobacillus bulgaricus* W + *Streptococcus thermophilus* H.

Table 3: Development of Acidity for Chakka and Shrikhand in terms of % LA during refrigerated Storage (5 ±2⁰C)

Days of storage	Type of culture used	Chakka	Shrikhand
		Refrigerated (5 ±2 ⁰ C)	Refrigerated (5 ±2 ⁰ C)
0	S ₁	0.896	1.213
	S ₅	1.860	1.303
5	S ₁	-	-
	S ₅	-	-
7	S ₁	-	-
	S ₅	-	-
10	S ₁	1.63	1.253
	S ₅	1.94	1.321
15	S ₁	1.96	1.256
	S ₅	1.94	1.336
20	S ₁	2.63	1.258
	S ₅	2.70	1.366
25	S ₁	-	-
	S ₅	-	-
30	S ₁	-	1.289
	S ₅	-	1.396
35	S ₁	-	-
	S ₅	-	-
40	S ₁	-	1.304
	S ₅	-	1.500
45	S ₁	-	-
	S ₅	-	-
50	S ₁	-	1.308
	S ₅	-	1.600
60	S ₁	-	-
	S ₅	-	-
Combined Mean (S ₁ + S ₅)		1.979	1.335
C. D.		0.239	0.012

* p < 0.05

S₁ – *Lactococcus lactis* sub sp. *cremoris* R₆.

S₅ – *Lactobacillus bulgaricus* W + *Streptococcus thermophilus* H

Conclusion

The study highlights the effectiveness of fermentation techniques in the preservation of milk, particularly for producing shrikhand, a popular fermented milk product. The selection of suitable starter cultures, such as *Lactococcus lactis* sub sp. *cremoris* R6 and a combination of *Lactobacillus bulgaricus* W with *Streptococcus thermophilus* H, proved essential for improving the product's acidity development, microbial quality, and shelf life. The findings suggest that modern technological applications can enhance both the safety and marketability of shrikhand, ensuring its widespread acceptance and consumption, while also supporting the growing fermented milk industry in India.

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