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Shelf Stability of Optimized Dahi Prepared using Functionally Active Lactic Cultures

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ABSTRACT

Background: Subjecting the optimized dahi prepared using functionally active lactic cultures.

Methods: During the period from 2012 to 2013, the wild strains of lactic isolates such as *L. lactis* ssp. *lactis* LC14 producing lactic acid; diacetyl and slime producing *L. mesenteroides ssp. mesenteroides* Leu8, probiotic nature (acid and bile tolerant) of *S. thermophilus* ST3 and only bile resistant *L. delbrueckeii* ssp. *bulgaricus* Lb32 were selected as they set the milk in 18 hours with good DMC and reasonable LA% for dahi preparation.

Result: The above mentioned LAB were combined for dahi preparation at 1% inoculum of each of 0.25% showed titratable acidity of 0.63 and DMC of 8.32 with 1 ppm of diacetyl; 180 ppm of exopolysaccharide or slime with sensory score of 7.6. The optimized dahi can be called as probiotic dahi due to addition of probiotic culture *S.thermophilus* ST3 along with other cultures. Upto 5 days and 11 days dahi could be stored at room temperature (24°C) and refrigeration temperature (7°C) was in good condition with acceptable quality respectively.

Key words: Diacetyl, Exopolysaccharide, Lactic acid, Lactic acid bacteria, Probiotic nature.

INTRODUCTION

Dahi is a thick, sour, well known fermented milk product of Indian house hold having heterogeneous lactic acid bacteria (LAB). *Lactococci, Streptococci, Leuconostoc, Pediococci, Lactobacilli* belong to LAB family. Normally the characteristics of the LAB that is required in a good quality dahi are the production of lactic acid, diacetyl, exopolysaccharide and tolerance to acid and bile. Lactic acid production sets the milk, inhibits spoilage and putrefactive bacteria, diacetyl is a flavouring agent (Garabal *et al.,* 2008), exopolysaccharide as prebiotic stimulating probiotic bacteria (Naessens *et al*., 2005) with acid and bile tolerant indicated the probiotic nature (Tannock, 1999) of the lactic culture. Based on an understanding of the culture's functionality, it is possible to select the most suitable strain or combination of strains. The systematic information on the natural microbial population present in fermented dairy products and/ or the mixed strain LAB cultures used commercially can help in formulation of defined strain starters with predictable performance for industrial use. Samanta *et al*. (2015) prepared curd using pasteurized Amul Milk with inoculum of 1% *Lactobacillus casei* (MTCC1408), *L. fermentum* (MTCC 903), *L. rhamnosus* (MTCC1462), *L. ingluviei* ADK10 (GenBank Accession No-JQ395039), *Enterococcus faecium* ADK18 (KF032592.1), *E. durans* ADK14. (KF032593.1) and *Streptococcus thermophillus* (MTCC 1938) separately with incubation at 37°C that revealed titratable acidity that ranged from 0.81 to 1.71% lactic acid in which high acidity (1.71%) was exhibited by *L. rhamnosus* while *S. thermophilus* exerted medium acidity of 0.81.

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MATERIALS AND METHODS

Maintenance of dahi cultures

Lactic cultures used in the preparation of dahi are the wild strains of lactic isolates such as *L. lactis* ssp. *lactis* LC14 producing lactic acid; diacetyl and slime producing *L. mesenteroide sssp. Mesenteroides* Leu8, probiotic nature (acid and bile tolerant) of *S. thermophilus* ST3 and only bile resistant *L. delbrueckii* ssp. *bulgaricus* Lb32 were maintained in sterile yeast glucose chalk litmus milk (Harrigan, 1998) as stock culture and sterile skim milk as working culture individually.

Optimization of dahi preparation

Dahi was prepared by using pasteurized homogenized milk obtained from student experimental dairy. The milk was heat treated for 85°C for 30 min, cooled to room temperature, inoculated with individual and combination of lactic culture

(*L. lactis ssp. lactis* LC14, *Streptococcus thermophilus* ST3, *L. mesenteroides ssp. mesenteroides* Leu8 and *L. delbrueckii* ssp. *bulgaricus* Lb32) at 1, 1.5, 2.0 and 2.5 per cent and incubated at 30°C till curd formation. The optimized dahi sample was subjected to sensory evaluation through 9 point hedonic scale, DMC, titratable acidity, diacetyl and exopolysaccharide estimation.

Analysis of optimized dahi

Sensory analysis

The sensory analysis was carried out by serving samples of dahi to a panel of judges with score card (9 point hedonic scale for dahi) to judge the quality of product with respect to colour and appearance, body and texture, flavour and overall acceptability. The scores given by panel of judges were then statistically analyzed.

Direct microscopic count (DMC), titratable acidity (TA), diacetyl and exopolysaccharide (EPS) estimation of dahi sample

Dahi samples were subjected to determination of DMC and TA as per standard procedure (IS:SP:18, 1981). Diacetyl estimation in dahi was carried out by using standard procedure (Garabal *et al*., 2008). Exopolysaccharide (EPS) or slime estimation was done using glucose as a standard (Garvie, 1986 and Torino *et al.,* 2001).

Shelf life study of the optimized dahi at ambient temperature and refrigeration

Dahi prepared was stored in retail cups at ambient temperature (24°C) and refrigeration temperature (5°C). Every day stored dahi was subjected to titratable acidity, viable count of total lactic acid bacterial count by serial dilution and pour plating using yeast glucose agar and sensory changes that occurred during the storage till the defect was visualized.

RESULTS AND DISCUSSION

Optimizing the dahi culture formulation for preparation of dahi

The selection criteria adopted for lactic cultures for preparation of dahi is lactic acid production, biomass and functional properties. Based on this, cultures such as *L. lactis* ssp. *lactis* LC14 that produced lactic acid and showed tolerance to bile; *Streptococcus thermophilus* ST3 that had probiotic nature (acid and bile tolerance); diacetyl and exopolysaccharide production by *L. mesenteroides* Leu8 and *L. delbrueckeii* ssp. *Bulgaricus* Lb32 showing lactic acid production and also had tolerance to bile were selected. Only the combination of cultures were used for dahi preparation as dahi contains heterogeneous microflora. The cultures were inoculated into heat treated (85°C/30 min.) homogenized milk at the rate of 1, 1.5 2.0 and 2.5 per cent at equal volume and incubated at 30°C till the milk was set. It was interesting to know that all the inoculum curdled the milk at 18 hours while dahi prepared with 1 per cent showed better organoleptic quality, DMC of 8.32 and TA of 0.63%. Dahi prepared using 1.5% and 2.0% showed syneresis which is a defect normally seen in dahi (Table 1). Dahi prepared using 1% inoculum (*L. lactis* ssp. *lactis* LC14 + *S. thermophilus* ST3 + *L. mesenteroides* Leu8 and *L. delbrueckeii* ssp. *bulgaricus* Lb32) contained 1ppm of diacetyl and 180 ppm of exopolysaccharides with sensory score of 7.6 (Table 2). Hence in further study, 1% inoculum was used in the preparation of dahi added to that, 1% liquid

Table 1: Optimizing the preparation of dahi.

Combination cultures used - (LC14+ ST3+ Leu8+ Lb32)	Setting time (hrs)	Titratable acidity (% LA)	DMC (log_{10}/ml)	DMC (Acidity) CD
1.0%	18	0.63	8.32	0.026 (0.013)
1.5%*	18	0.66	8.28	
2.0%**	18	0.66	8.25	
2.5%***	18	0.72	8.20	

- Combination of cultures used for dahi preparation was *L. lactis ssp. lactis* LC14 + *Streptococcus thermophilus* ST3 + *L. mesenteroides ssp. mesenteroides* LEU8 + *L. delbrueckii* ssp. *bulgaricus* LB32.
- *Showed syneresis.
- **Showed more syneresis.

Table 2: Characteristics of optimized dahi.

Combination of cultures used	Setting time (hrs)	Sensory score (9)	Titratable acidity (% LA)	DMC (log_{10} /gm)	Diacetyl (ppm)	Exoploysaccharide (ppm)
LC14 + ST3 + LEU8 + LB32	18	7.6 (Overall acceptability)	0.63	8.32	1.0	180

- The values are the average of three trials.
- Combination of cultures used for dahi preparation was *L. lactis ssp. lactis* LC14 + *Streptococcus thermophilus* ST3 + *L. mesenteroides ssp. mesenteroides* LEU8 + *L. delbrueckii* ssp. *bulgaricus* LB32.

Table 3: Effect of storage on titratable acidity and viable count of lactic cultures in optimized dahi at room temperature (24°C) and refrigerated temperature (7°C).

Day of storage	Room temperature stored titratable acidity (% LA)	Viable count of LAB (log_{10}cfu/gm)	Refrigerated temperature stored titratable acidity (% LA)	Viable count of LAB (log_{10}cfu/gm)
0	0.63	8.12	0.63	8.12
1	0.64	8.12	0.63	8.03
2	0.64	7.88	0.64	7.98
3	0.64	7.42	0.64	7.83
4	0.64	7.31	0.64	7.75
5	0.64	7.15	0.64	7.53
6	Spoiled by mold growth		0.64	7.45
7	on the surface		0.64	7.32
8			0.64	7.28
9			0.64	7.12
10			0.64	6.98
11			Spoiled by mold growth on the surface	

- The values are average of three trials.
- Combination of cultures used for dahi preparation was *L. lactis ssp. lactis* LC14 + *Streptococcus thermophilus* ST3 + *L. mesenteroides ssp. mesenteroides* LEU8 + *L. delbrueckii* ssp. *bulgaricus* LB32.

culture is comfortable for bulk preparation of dahi culture. The optimized dahi can be called as probiotic dahi due to incorporation of probiotic culture *S. thermophilus* ST3 in dahi preparation along with other cultures.

In order to suit the Indian house hold conditions for dahi preparation during summer with ambient temperature of 34°C and 37°C nearly a related study by Mogra and Choudhry (2008) who found combinations of *S. diacetylactis* + *L. bulgaricus* (2% at 34°C) and *S. lactis* + *S. diacetylactis* + *S. thermophillus* (3% at 37°C) as best cultures.

Storage study of dahi culture by selective isolates at room temperature (24°C) and refrigeration temperature (7°C)

With 1% inoculum of *L. lactis* ssp. *lactis* LC14,*Streptococcus thermophilus* ST3, *L. mesenteroides* Leu8 and *L. delbrueckeii* ssp. *bulgaricus* Lb32, dahi was prepared using homogenized milk and incubated at 30°C/18 hours. The prepared dahi was stored at room temperature (recorded as 24°C) and subjected to titratable acidity and viable count of total LAB using yeast glucose agar. On 0 day, after the setting of milk, acidity of dahi was 0.63% with viable count of 8.12 log_{10}cfu/g, later days showed reduction in viable count (7.15 log_{10}cfu/g) by 1 log number and acidity maintained as 0.62%. After 5th day of storage, mold growth was observed on the surface indicating defect in dahi and hence discarded. Dahi prepared can be stored for 4 days for use without increase in acidity and acceptable microbiological quality.

Dahi prepared using 1 per cent inoculum was stored at refrigeration temperature (recorded as 7°C) and subjected to titratable acidity and viable count of total LAB using yeast glucose agar. The titratable acidity of dahi was 0.63% with viable count of 8.12 log_{10}cfu/g was noticed once dahi was prepared. As storage period increased, on 9th day the viable count of LAB reduced by 1 log count. After 11th day of storage mold growth was noticed on the surface indicating defect in dahi. The reduction in viable count may be attributed to the acidity produced and low storage temperature. Dahi prepared can be stored for 10 days for use in refrigerator (7°C) without increase in acidity and acceptable microbiological quality (Table 3).

On the contrary, the study conducted by Bire and Pande (1983), curd could be stored at room temperature (24°C) safely without any spoilage and without development of foul smell for 7 days with constant acidity of about 1.85%. On par with the present study, Kamruzzaman *et al.* (2002) showed good condition of dahi up to 3 days of storage period at room temperature and 12 days at refrigeration temperature.

CONCLUSION

Dahi prepared using 1% inoculum (*L. lactis* ssp. *lactis* LC14 + *S. thermophilus* ST3+ *L. mesenteroides* Leu8 and *L. delbrueckeii* ssp. *bulgaricus* Lb32), contained 1 ppm of diacetyl and 180 ppm of exoploysacharides with sensory score of 7.6. The optimized dahi can be called as probiotic dahi due to addition of probiotic culture *S. thermophilus* ST3 along with other cultures. Upto 4 days and 10 days dahi stored at room temperature and refrigerated temperature was in good condition with acceptable quality respectively.

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