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Original article

Selection of starter culture for production of indigenous fermented milk product *(Misti dahi)*

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Summary — *Misti dahi* is a traditional Indian fermented milk product prepared from partially concentrated sweetened milk. Besides variations in chemical and microbiological qualities the market survey of *Misti dahi* has revealed many flavour defects such as fruity, alcoholic, acidic or flat taste and textural defects such as gassiness, weak body, wheying-off and dried surface. Since this product is gaining commercial significance, the technology for its industrial production has been developed. Eight lactic cultures, *viz S lactis* (C_{10}), *S diacetylactis* (DRC_1); *S cremoris* (C_1); *S thermophilus* (HST); *L bulgaricus* (LBW); 1:1 mixture of C_{10} and DRC_1; 1:1 mixture of HST and LBW; LF-40 (a mixed culture of different strains of *S lactis, S diacetylactis, S cremoris* and *Leuconostoc*) were examined for their ability to grow in a concentrated, sweetened milk system (18% milk solids and 14% sucrose). On the basis of lactic acid, diacetyl and acetyl methyl carbinol production and pH and curd tension measurements, the LF-40 culture was found to be the most suitable for commercial production of *Misti dahi*.

starter culture / *Misti dahi* / sweetened fermented milk

Résumé — Sélection de levain pour la production d'un produit laitier fermenté indien *(Misti Dahi).* *Le* Misti dahi *est un produit laitier fermenté traditionnel indien préparé à partir de lait sucré partiellement concentré. Un aperçu du marché du* Misti Dahi, *révèle, outre des variations dans la qualité chimique et microbiologique, plusieurs défauts de flaveur (fruitée, alcoolisée, acide, fade) et de texture (gazeuse, fragile, exsudante, à la surface desséchée). L'importance commerciale que prend ce produit a nécessité le développement d'une technologie adaptée à une production industrielle. Huit cultures lactiques :* S lactis *(C_{10}),* S diacetylactis *(DRC_1),* S cremoris *(C_1),* S thermophilus *(HST),* L bulgaricus *(LBW), mélange 1:1 C_{10} et DRC_1, mélange 1:1 HST et LBW, culture mixte de plusieurs espèces de* S lactis, S diacetylactis, S cremoris *et* Leuconostoc *(LF-40), ont été examinées pour leur capacité à croître dans un lait concentré sucré (18% de matière sèche et 14% de sucrose). Sur la base de la production d'acide lactique, de diacétyle et d'acétyle méthyle carbinol, des valeurs de pH et de fermeté du gel obtenues, la culture LF-40 s'est avérée être la plus apte à la production industrielle de* Misti Dahi.

levain* / Misti Dahi / *lait sucré fermenté

INTRODUCTION

Misti dahi (syn sweetened curd, *lal dahi, payodhi*) is a sweetened fermented milk product from the eastern part of India. It is prepared on cottage scale to cater to local demand. Traditionally, milk with cane sugar is heated in an open pan at simmering temperature (60–70 °C) for 6–7 h to evaporate part of the moisture and to develop a marked cooked flavor, slightly brown color, viscosity and other physico-chemical

changes. After cooling to ≈ 30 °C, the mix is inoculated with commercial starter culture kept from the previous day, and transferred to earthenware pots; curdling takes place at room temperature overnight. The confectioners who sell this product do not adopt preventive measures for maintaining the reliability and purity of culture. Wide variations in total solids content (27–43 %), non fat milk solids (11–16%) and sucrose (13–19%) in the market samples of *Misti dahi* sold in Calcutta have been reported by Ghosh and Rajorhia (1987). Flavour defects such as fruity, alcoholic, highly acidic, flat taste and textural defects such as gassiness, weak body, wheying-off and thick crust on top were observed in most of the market samples. In view of the increasing nationwide demand for *Misti dahi* and the growing interest on the part of the organized dairies for large-scale manufacture, there is an urgent need for modernizing the technology for its commercial production. In this communication, investigation related to selection of a suitable starter culture capable of growing in partially concentrated milk especially in the presence of sucrose are reported. The physico-chemical and sensory properties of *Misti dahi* as influenced by the starter culture are also described.

MATERIALS AND METHODS

Milk

Fresh raw milk was procured from a buffalo herd maintained at the National Dairy Research Institute, Karnal, India.

Starter cultures

The pure cultures of *Streptococcus lactis* (C_{10}), *Streptococcus diacetylactis* (DRC_1), *Streptococcus cremoris* (C_1), *Streptococcus thermophilus* (HST), *Lactobacillus bulgaricus* (LBW) and LF-40 (a patented culture of mixture of different strains of *S lactis*, *S diacetylactis*, *S cremoris* and *Leuconostoc*) were obtained from the Centre for National Collection of Dairy Microorganisms of the Institute and aseptically activated in the laboratory. Active cultures of C_{10} and DRC_1 were mixed in equal proportions before propagation. Likewise, a 1:1 mixture of HST and LBW was also prepared.

Preparation of Misti dahi

Fresh buffalo milk was standardized to 3.5% fat and 9.0% SNF (solid non fat), heated to 65 °C (with no hold) in a plate heat exchanger and homogenized at 56 kg/cm^2 (one stage). Milk was concentrated 1.44-fold in a presterilized single effect evaporator with a vacuum of 63.5–66.4 cm mercury. To this concentrate, dry cane sugar was added at the rate of 14.0%. The resultant mix was heated in a closed stainless steel container at 85 °C for 10 min to generate a cooked flavour and slight brown colour. The mix was water-cooled to 42 °C (for thermophilic cultures) and 30 °C (for mesophilic cultures) before inoculation with experimental cultures. The inoculated mix was aseptically distributed into presterilized polystyrene containers (100 ml) and transferred to incubation chambers at 30 °C for 7 h for mixes containing the cultures of C_{10}, C_1, DRC1, LF-40 and 1:1 mixture of C_{10} and DRC_1, while those mixes containing cultures of HST, LBW and mixtures of HST and LBW were incubated at 42 °C for 4 h. After incubation, the samples were shifted to cold store and maintained at 4 °C.

Titratable acidity (TA)

The method described in Indian standard (1981) part XI was used for TA determination. About 10 g of sample was weighed and mixed with 10 ml of CO_2-free distilled water. The contents were titrated with 0.1 N sodium hydroxide solution in the presence of 0.5% (w/v) phenolpthalein indicator. TA was calculated as the percentage of lactic acid in the product. TA in the range of 0.8–0.85% was considered optimum in *Misti dahi*.

pH

A digital pH meter, model L1-122 (Elico Pvt Ltd, India) was calibrated with standard buffer solution at 30 °C. Samples at 30 °C were rendered into homogenous paste with pastle and mortar and tested for pH by direct immersion of electrode in the sample.

Curd tension

Curd tension was measured by the method of Chandrashekhara *et al* (1957) with a slight modification, as suggested by Patel and Chakraborty (1985). A 50-ml portion of mix was placed in a 100-ml glass beaker (7 x 4.5 cm) and a H-shaped blade placed in it. Mixes were than inoculated with the experimental cultures. Mixes containing C_1, C_{10}, DRC_1, LF-40 and a mixture of C_{10} and DRC_1 were incubated at 30 °C for 7 h, while other mixes containing cultures of HST, LBW and a mixture of HST and LBW were incubated at 42 °C for 4 h. At the end of the incubation period, the weight (in g) required to pull the knife out of the curd was recorded.

Diacetyl and acetyl methyl carbinol (AMC)

The rapid colorimetric method recommended by King (1948) was used. Intensity of pink colour was measured at 530 nm in a photoelectric colorimeter, model AE 11 (Erma, Japan). The value of diacetyl and acetyl methyl carbinol were calculated from the standard curve.

Sucrose tolerance test

To 20 ml of fresh buffalo skim milk (9% SNF, 0.1% fat) in the test tubes different amounts of sucrose were added so that the concentration in the test tubes was 2, 6, 8, 10, 12, 14 and 16%. The contents were sterilized in an autoclave at 121 °C for 15 min and after cooling inoculated with the experimental cultures at the rate of 1.0%. A control sample without sucrose was also run simultaneously. The tubes containing the cultures of C_1, C_{10}, DRC_1, LF-40 and mixture of C_{10} and DRC_1 were incubated at 30 °C for 7 h while the tubes containing cultures of HST, LBW and a mixture of HST and LBW were incubated at 42 °C for 4 h. After incubation, the contents of the tubes were tested for TA.

Sensory evaluation

The samples of *Misti dahi* were subjected to sensory evaluation by a panel of 7 trained judges, using a 9-point hedonic scale ("most liked", 9 points; to "most disliked", 1 point).

RESULTS AND DISCUSSION

Sucrose tolerance of starter cultures

The TA developed in the presence of varying concentrations of sucrose in skim milk by different strains of starter cultures are presented in table I. The initial TA of skim milk was 0.13%. The rate of TA production decreased with an increasing level of sucrose for all the experimental cultures except for HST up to 6% level of sucrose. In mesophiles, C_{10} showed the maximum tolerance to sucrose in the range of 6–16% concentration. Lactic acid formation by DRC_1 and C_1 cultures was retarded with the progressive sucrose concentration. The inhibitory effect of higher concentrations of sucrose was more pronounced in DRC_1 culture. Starter culture LF-40 showed higher tolerance to sucrose than other mesophilic cultures except C_{10}. The mixed culture of DRC_1 and C_{10} (1:1) was the least effective of the mesophilic cultures in TA production.

In the thermophilic cultures, HST produced a higher percentage of acid in the presence of sucrose up to 6% than the control. *S thermophilus* is capable of producing lactic acid from sucrose (Deibel and Seeley, 1974). Beyond the 8% level the

Table I. Activity of starter cultures in skimmed milk in presence of sucrose. Figures represent the means of 3 trials.
Activité des levains dans du lait écrémé en présence de sucrose.

Sucrose concentration (%)	*Types of culture*							
	LF-40	*DRC_1*	*C_{10}*	*C_1*	*DRC_1:C_{10}(1:1)*	*HST*	*LBW*	*HST:LBW(1:1)*
	Percent lactic acid							
Control	0.55	0.48	0.43	0.41	0.40	0.56	0.39	0.91
2	0.53	0.48	0.40	0.40	0.40	0.57	0.36	0.89
4	0.53	0.46	0.40	0.38	0.39	0.59	0.34	0.89
6	0.51	0.44	0.41	0.38	0.37	0.59	0.31	0.88
8	0.50	0.40	0.40	0.37	0.37	0.56	0.28	0.85
10	0.48	0.39	0.39	0.35	0.38	0.55	0.25	0.81
12	0.48	0.37	0.38	0.34	0.31	0.54	0.21	0.78
14	0.44	0.33	0.38	0.32	0.31	0.49	0.18	0.76
16	0.41	0.30	0.37	0.31	0.29	0.46	0.16	0.73

rate of acid production by HST gradually decreased. *L bulgaricus* (LBW) produced maximum acid in the control samples, but activity was severely inhibited with an increasing level of sucrose in skim milk. It has been clearly demonstrated that *S thermophilus* has a greater tolerance to sucrose than *L bulgaricus* (Ray and Srinivasan, 1972; Tramer, 1973). Lactic acid production proceeded more rapidly with mixed cultures (1:1) of HST and LBW than those used singly. The symbiotic relationship of HST and LBW was clearly observed in the control samples. Their activity was not significantly affected up to 6% sucrose concentration. However, with further increase in sucrose concentration, the acid production declined, possibly due to the inhibition of *Lactobacillus* organisms (Tramer, 1973).

Performance of starter culture in production of Misti dahi

The initial acidity and pH of mix at the time of inoculation were not affected by the types of cultures used (tables II, III). The TA and pH at curd setting stage were significantly influenced by the type of organism. In mesophilic cultures, LF-40 and C_{10} were found to produce more acidity than the other cultures (table II). DRC_1 produced more acid than C1 (table III). The low TA with mixed starter culture (DRC_1 and C_{10}) may be attributed to the non-symbiotic nature of the 2 cultures, as suggested by Patel *et al* (1983). On the other hand, thermophilic culture showed rather interesting results. Culture HST produced acid at a faster rate than the LBW culture (table III) as the former retains its activity even at a higher sucrose concentration of 10–12% (Marshall and Mabbitt, 1980).

The activity of the *Lactobacillus* cultures was greatly affected with the increase in sucrose concentration which alters morphology by elongating and distorting the cells (Tramer, 1973). However, the mixed culture of HST and LBW showed excellent performance in terms of the highest level of acid production in the presence of sucrose. However, this combination was not found suitable for flavor production in *Misti dahi*.

Table II. Effect of different cultures on physico-chemical and sensory properties of *Misti dahi.* Figures in parentheses are the means of 3 trials. Inoculation rate : 1.0% for all cultures.
Effet des différentes cultures sur les propriétés physico-chimiques et sensorielles du Misti dahi.

Characteristics		*Type of culture*			
		LF-40	*C_{10}*	*DRC_1*	*DRC_1 :C_{10} (1:1)*
Acidity (% LA)	Initial	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)
	Final	0.85 – 0.86 (0.85)	0.81 – 0.83 (0.82)	0.79 – 0.80 (0.80)	0.75 – 0.78 (0.76)
pH	Initial	6.38 – 6.41 (6.39)	6.38 – 6.41 (6.39)	6.38 – 6.41 (6.39)	6.38 – 6.41 (6.39)
	Final	4.49 – 4.51 (4.50)	4.53 – 4.55 (4.54)	4.58 – 4.60 (4.59)	4.68 – 4.70 (4.69)
Diacetyl + AMC (µg/g)		19 – 21 (20)	0.00	23 – 24.5 (23.5)	16 – 17.5 (16.8)
Average aroma score (maximum 9)		7.78	5.50	6.76	7.57
Curd tension (g)		25 – 28 (26.3)	24 – 27 (25.3)	22 – 24 (23.0)	18 – 19 (18.3)
Average curd firmness score (maximum 9)		7.95	7.75	7.59	7.28

The production of flavor volatiles was found to be affected by the type of microorganism. DRC_1 produced the highest amount of flavor volatiles followed by LF-40 and a mixed culture of DRC_1 and C_{10}. The C_{10} failed to produce diacetyl and AMC even after 7 h of incubation at 30 °C. The C_{10} culture is inferior to C_1 from the standpoint of flavor volatile production (Dutta *et al,* 1971). In the thermophilic cultures, HST produced the highest amount of diacetyl and AMC (table III). *S thermophilus* is capable of producing flavor volatiles in milk at low concentrations (Bottazzi and Vescovo, 1969). The mixed culture produced the least amount of flavor volatiles (Bottazzi *et al,* 1971; Yu and Nakanishi, 1975). In our experiment, LBW culture did not perform well in flavor production in the presence of sucrose. The relative capacity of the microorganisms to ferment citrate differs considerably (Cogan, 1982). The aroma scores of *Misti dahi* prepared with LF-40 culture were the highest, followed by mixed culture (DRC_1 and C_{10}) and DRC_1 (table II). *Misti dahi* prepared with C_{10} had the lowest aroma scores. Other cultures like C_1, HST, LBW and mixed culture (HST and LWB) also received a very low flavor grading due to production of insufficient amounts of diacetyl and AMC. These volatiles are most important in imparting a characteristic aroma to *Misti dahi.* The changes in the aroma scores were found to be significant at $P < 0.01$. Aroma and taste are jointly important for

Table III. Effect of different cultures on physico-chemical and sensory properties of *Misti dahi.* Figures in parentheses are the means of 3 trials. Inoculation rate = 10% for all cultures.
Effet des différentes cultures sur les propriétés physico-chimiques du Misti dahi.

Characteristics		*Type of culture*			
		C_1	*HST*	*LBW*	*HST: LBW (1:1)*
Acidity (% LA)	Initial	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)
	Final	0.75 – 0.76 (0.75)	0.85 – 0.87 (0.86)	0.61 – 0.63 (0.62)	0.90 – 0.93 (0.92)
pH	Initial	6.38 – 6.41 (6.39)	6.38 – 6.41 (6.39)	6.38 – 6.41 (6.39)	6.38 – 6.41 (6.39)
	Final	4.68 – 4.70 (4.69)	4.48 – 4.51 (4.50)	4.84 – 4.87 (4.85)	4.39 – 4.42 (4.41)
Diacetyl + AMC (µg/g)		2 – 3 (2.0)	5 – 7 (5.6)	2.5 – 3.5 (3.0)	2 – 3 (2.3)
Average aroma score (maximum 9)		5.61	6.30	5.88	5.73
Curd tension (g)		18 –20 (19)	26 – 28 (27.3)	12 – 13 (12.3)	28 – 30 (29.0)
Average curd firmness score (maximum 9)		7.31	7.91	6.30	8.13

consumer acceptability of *Misti dahi.* In spite of the highest amount of diacetyl and AMC produced by DRC_1, *Misti dahi* samples scored low flavor ratings because of the hetero-fermentative nature of the bacterial culture. DRC_1 is known to produce small amounts of formic acid and CO_2 which imparts a sharp taste to the product.

The firmness of the curd formed by the lactic culture depends on the pH at the time of coagulation. Gel formation is strongly supported by low pH in fermented milk, especially when milk receives high heat treatment leading to casein–whey protein interaction (Perry, 1974). The "most liked" curd firmness in *Misti dahi* was produced by mixed culture (1:1) of HST and LBW (table III) followed by LF-40 (table II) and HST. Other cultures received lower firmness ratings. The differences in the average curd firmness scores were found to be statistically significant at $P < 0.01$.

As the type of culture employed in the preparation of Indian fermented milk products depends upon the desired amount of acidity, flavor volatiles and optimum curd firmness, LF-40 culture proved to be most useful. Further studies were therefore conducted on LF-40 culture.

Effect of inoculum levels of LF-40

The flavor volatiles increased in the product with increasing percentage of inoculum, apparently as a result of higher citrate

Table IV. Influence of LF-40 inoculum on physico-chemical and sensory properties of *Misti dahi.* The figures in paentheses are the means of 3 trials.
Influence de l'inoculation de LF-40 sur les propriétés physico-chimiques et sensorielles du Misti dahi.

Characteristics		*Percent inoculum*			
		0.5	*1.0*	*1.5*	*2.0*
Acidity (% LA)	Initial	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)	0.28 – 0.29 (0.28)
	Final	0.72 – 0.75 (0.74)	0.78 – 0.80 (0.79)	0.80 – 0.84 (0.82)	0.88 – 0.90 (0.89)
pH	Initial	6.32 – 6.35 (6.33)	6.32 – 6.35 (6.33)	6.32 – 6.35 (6.33)	6.32 – 6.35 (6.33)
	Final	4.62 – 4.65 (4.63)	4.53 – 4.61 (4.57)	4.49 – 4.56 (4.53)	4.42 – 4.49 (4.46)
Diacetyl + AMC (µg/g)		18 – 19 (18.6)	20 – 21 (20.3)	22 – 25 (23.3)	25 – 27.5 (25.7)
Average aroma score (maximum 9)		6.86	7.88	7.38	7.09
Curd tension (g)		20 –22 (21.3)	28 – 29 (28.3)	25 – 27 (25.6)	21 – 23 (22.0)
Average curd firmness score (maximum 9)		6.71	8.05	7.83	7.24

utilization (Tiwati *et al,* 1972). The flavor scores were maximum in the case of samples prepared with 1.0% of inoculum. Higher doses of LF-40 inoculum produced a sharp taste, leading to low sensory ratings. The differences in flavor scores were found to be statistically significant at $P < 0.01$. On the basis of critical difference (0.39), 1.0 and 1.5% inoculum would give a product with almost similar quality attributes (table IV).

The firmness of *Misti dahi* was influenced by the percentage of inoculum. Amongst 4 dosages, 1.0% produced the best results as judged by curd tension readings. The average firmness scores were the highest in samples prepared with 1.0% inoculum. The differences in the average scores due to amount of inoculum were significant at $P < 0.01$ (table IV). Addition of 2.0% inoculum resulted in a slight weak body and coarse texture due to a high rate of acidification during incubation which might favor a very dense aggregation of protein particles with a corresponding decrease in bound water (Rasic and Kurmann, 1978).

CONCLUSION

It is concluded that LF-40 culture (mixture of various strains of *S lactis, S diacetylactis, S cremoris* and *Leuconostoc*) is most

appropriate for commercial production of *Misti dahi* from buffalo milk containing ≈ 18% milk solids and 14% sucrose.

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