

EVALUATION OF EXOPOLYSACCHARIDES (EPS) AND NONEXOPOLYSACCHARIDES (NON EPS) PRODUCING STRAINS OF LACTIC ACID BACTERIA TO SELECT THE BEST COMBINATIONS OF CULTURES FOR DAHI PREPARATION

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Abstract: The present study was carried out to investigate the changes in physicochemical and sensory properties of milk fermented with different exopolysaccharides (EPS) and nonexopolysaccharides (non EPS) producing strains of lactic acid bacteria. Different cultures procured from market and the Department of Dairy Microbiology, were used to prepare *Dahi*. All the curd samples were prepared from milk standardized to 3.5% fat and 14% TS. Standardized milk was fermented at temperature of 39°C till the pH reached up to 4.6 followed by transfer of samples to refrigerator ($5 \pm 2^\circ\text{C}$) for overnight. Sensory analyses were carried out next day morning. In screening phase, fifteen lactic cultures of *Lactococcus lactis*, *Streptococcus thermophiles* and *Lactobacilli* nomenclatured from A to M were analysed for sensory analysis. The results for sensory attributes revealed that cultures B (8.25), D (8.00), K (8.09) and L (8.07) were better in terms of overall acceptability. Total eleven culture combinations were made on the basis of screening phase to obtain a better quality *Dahi*. Different combinations were A2 (B + E), B2 (B + I), C2 (D + E), D2 (D + I), E2 (J + L) and F2 (M + K) in a ratio of 50:50, while G2 (B + E), H2 (B + I), I2 (D + E) and J2 (D + I) in a ratio of 75:25. The *Dahi* prepared with cultures E2 (8.00), F2 (7.95), I2 (8.21) and J2 (8.35) showed maximum acceptability as reflected by highest scores for different parameters during sensory evaluation.

Keywords: Physicochemical, Sensory, Exopolysaccharides, Lactic acid bacteria, *Dahi*.

INTRODUCTION

Indian curd known as “*Dahi*” is a very well-known fermented milk product resembling yoghurt consumed by large sections of the population throughout the country, either as a part of the daily diet or as a refreshing beverage (Prajapati and Gawai, 2011). Since conversion of milk into *Dahi* is an important intermediately steps in manufacture of indigenous fat-rich dairy products like butter and ghee, it can be said that over 40% of the total milk production in India is converted into *Dahi*.

Received Sep 16, 2016 * Published Oct 2, 2016 * www.ijset.net

As per the FSSAI rule (2011), *Dahi* or curd the product obtained from pasteurized or boiled milk by souring, natural or otherwise, by a harmless lactic acid culture or other harmless bacterial culture may also be used in conjunction with lactic acid bacteria culture for souring. Good qualities *Dahi* is of firm and uniform consistency with a sweet aroma and clean acid taste (German *et al.*, 1999). The molecular mass of these polymers ranges between 4.0×10^4 and 6.0×10^6 Da. The heteropolysaccharides are constructed from multiple copies of oligosaccharides, which contain between three and eight residues. Commercial culture of Indian origin for *Dahi* making are also not available. So by this research we will be getting combinations of cultures which will be providing standard quality *Dahi*.

Keeping these facts in view, this investigation is envisaged to evaluate different EPS and non EPS producing strains of Lactic acid bacteria with an objective of selecting best combinations of EPS and non-EPS producing cultures for making quality *Dahion* the basis of physicochemical and sensory evaluation.

MATERIALS AND METHODS

The raw cow's milk for the production of *Dahi* was obtained from LRS, AAU, Gujarat, India. The milk contained 3.5% fat and 12% total solids. The acidity and pH of the milk were 0.126 ± 0.002 % LA, 6.65 ± 0.02 , respectively. The different strains used for culture combinations in the present study are indicated in Table 1 and 2.

Table 1: List of Strains for preparation of cultures

Species	Strain no.	Origin
<i>Streptococcus thermophiles</i>	ST417, ST11, ST684, ST13, ST32, ST757, ST614, ST115, ST303, ST505, ST503 and ST820	SACCO
	MD ₂ and MD ₈	SMC college of dairy science
<i>Streptococcus lactis</i>	SL216, SL69, SL225, SL204, SL56, SL228, SL119, SL197, SL195, SL220 and SL232	SACCO
<i>Lactobacillus helveticus</i>	MTCC 5463 (V ₃)	SMC college of dairy science
<i>Lactobacillus rhamnosus</i>	NS ₆	SMC college of dairy science

Table 2: Culture combinations used in present study

Culture code	Type of strains and code used in screening phase	Full strain name
A2	B (ST13+ST32+ST757) + E (SL216+SL69+SL225) (50:50)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>
B2	B (ST13+ST32+ST757) + I(SL119+SLSL195+SL232) (50:50)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>
C2	D (ST505+ST503+ST820) + E (SL216+SL69+SL225) (50:50)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>
D2	D (ST505+ST503+ST820) + I(SL119+SLSL195+SL232) (50:50)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>
E2	J (V ₃) + L (MD ₂) (50:50)	V ₃ = <i>Lactobacillus helveticus</i> MTCC 5463 MD ₂ = <i>Streptococcus thermophilus</i> MTCC 5460
F2	M (NS ₆) + K (MD ₈) (50:50)	NS ₆ = <i>Lactobacillus rhamnosus</i> MTCC 5946 MD ₈ = <i>Streptococcus thermophilus</i> MTCC 5461
G2	B (ST13+ST32+ST757) + E (SL216+SL69+SL225) (75:25)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>
H2	B (ST13+ST32+ST757) + I(SL119+SLSL195+SL232) (75:25)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>
I2	D (ST505+ST503+ST820) + E (SL216+SL69+SL225) (75:25)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>
J2	D (ST505+ST503+ST820) + I(SL119+SLSL195+SL232) (75:25)	ST= <i>Streptococcus thermophiles</i> SL = <i>Streptococcus lactis</i>

The cultures were propagated in sterile reconstituted skim milk (10% TS) at 39°C for 16 h and stored at 5 ± 2°C. Other cultures were obtained from SACCO, Italy, commercially available in DVS form. DVS cultures were stored at -20°C.

Chemical analysis of Milk / Dahi

Determination of Titratable Acidity: Titratable acidity of cow milk / Dahi was estimated by the procedure described in (IS: 1479, part I. 1960). Titratable acidity was calculated by the following formula: Acidity (as % lactic acid) = 9AN/W

Where, A = Volume in ml of 0.1N NaOH required for titration, N = Normality of NaOH solution, and W = Volume in ml of milk taken for the titration.

Determination of pH: pH of cow milk / *Dahi* was determined by electronic pH meter, Model CYBERSCAN series 600 waterproof portable meter manufactured by EUTECH Instruments, Singapore.

Sensory evaluation: The overall quality and acceptability of *Dahi* samples were assessed by a consumer oriented panel on day 1 for selection of best cultures. On the basis of 9 point hedonic scale, culture combinations were selected. Fresh product at 0 days and the stored products (7, 14, 21 days stored at 4°C) were brought to 10°C before giving for judging. The score given by them on 9 – point hedonic scale were taken to determine the acceptability level of product (Appendix 1).

RESULT AND DISCUSSION

Overall quality of fermented milk depends on sensory properties. Fermented milk products have a clean acid taste and a delicate aromatic flavor. It should be free from undesirable off flavors like fat, metallic, yeasty and bitter. The surface should be smooth and glossy, while the cut surface should be trim and free from cracks and gas bubbles (De, 1980; Bhimasena Rao and Dastur, 1955). Based on sensory evaluation cultures B, D, E, I, J, K, L and M were found to be optimum as compared to control and selected for carrying of the further analysis.

Changes in the Physico-Chemical Properties of *Dahi* Prepared from Different Combinations of Cultures

Dahi had a final pH of 4.5 to 5.0. Cultures A2, B2, G2 and H2 took 6 h and 30 min to reach upto pH 4.6, while cultures control, C2, D2, E2, F2, I2 and J2 took 6 h for coagulation. The data in indicated that per cent lactic acid content in all the cultures ($p < 0.05$). There was not much difference in acidity valued for all culture. All the cultures had a non-significant difference; they were at par.

Table 3: Change in pH and acidity of different *Dahi* samples

Cultures	Ph	Acidity (%LA)
Control	4.60 ± 0.01	0.73 ± 0.01
A2	4.60 ± 0.01	0.73 ± 0.01
B2	4.62 ± 0.008	0.73 ± 0.004
C2	4.61 ± 0.01	0.73 ± 0.02
D2	4.60 ± 0.02	0.72 ± 0.009
E2	4.60 ± 0.004	0.73 ± 0.014
F2	4.61 ± 0.01	0.73 ± 0.009

G2	4.61 ± 0.004	0.73 ± 0.009
H2	4.62 ± 0.008	0.74 ± 0.008
I2	4.62 ± 0.014	0.73 ± 0.01
J2	4.61 ± 0.02	0.73 ± 0.009
Average	4.61	0.73
SEm	0.01	0.001
CD (0.05)	0.04	0.01
CV%	0.46	0.34

Sensory Analysis of *Dahi* Prepared from Combination of Cultures

The sensory characteristics like color and appearance, flavour, body & texture, acidity and overall acceptability of *Dahi* samples were analyzed using a nine point hedonic scale. A panel of seven judges was selected for the analysis.

Table 4: Sensory score of *Dahi* prepared from combinations of cultures

Cultures	Flavour	Body	Acidity	Color and Appearance	Overall acceptability
Control	8.24±0.03 ^d	8.27±0.08 ^d	8.23±0.04 ^c	8.34±0.07 ^{bc}	8.25±0.05 ^f
A2	7.29±0.24 ^{ab}	7.94±0.05 ^{bc}	7.46±0.18 ^a	7.97±0.11 ^a	7.69±0.08 ^{abc}
B2	7.06±0.18 ^a	7.96±0.22 ^{bc}	7.40±0.22 ^a	7.99±0.11 ^a	7.62±0.13 ^{ab}
C2	7.45±0.03 ^b	8.25±0.06 ^d	7.27±0.02 ^a	8.07±0.06 ^{ab}	7.84±0.05 ^{cde}
D2	7.43±0.02 ^{ab}	7.97±0.05 ^{bc}	7.39±0.04 ^a	8.14±0.24 ^{abc}	7.76±0.03 ^{bcd}
E2	8.02±0.12 ^{cd}	8.07±0.12 ^{cd}	7.87±0.11 ^b	8.41±0.14 ^c	8.00±0.07 ^e
F2	7.86±0.24 ^c	8.11±0.13 ^{cd}	7.74±0.13 ^b	8.28±0.19 ^{bc}	7.95±0.05 ^{de}
G2	7.31±0.34 ^{ab}	7.61±0.05 ^a	7.36±0.19 ^a	8.10±0.17 ^{ab}	7.59±0.17 ^{ab}
H2	7.04±0.47 ^a	7.77±0.19 ^{ab}	7.29±0.24 ^a	8.00±0.00 ^a	7.52±0.21 ^a
I2	8.12±0.15 ^{cd}	8.2±0.017 ^d	8.1±0.16 ^c	8.35±0.21 ^{bc}	8.21±0.14 ^f
J2	8.25±0.07 ^d	8.270±0.05 ^d	8.250±0.08 ^c	8.39±0.15 ^c	8.35±0.06 ^f
Average	7.64	8.04	7.67	8.18	7.89
SEm	0.12	0.07	0.09	0.33	0.06
CD (0.05)	0.35	0.19	0.25	0.12	0.18
CV%	2.73	1.41	1.91	2.47	1.38

All data are mean of three independent replications

Values having different superscript within column differ significantly ($p < 0.05$)

Flavour and Body: It was observed that the flavour score was significantly higher for culture J2 (8.25), and followed by control, I2, E2 and F2. Flavour score for *Dahi* samples were ranging from 7.04 to 8.25 and average was 7.64. Cultures A2, B2, D2, G2 and H2 had non-significant difference. Cultures control, E2, I2 and J2 were at par and are in accordance with Yang *et al.* (2010). Culture J2 and control were significant and better in comparison to other cultures and average score for body was 8.04. Cultures A2, B2, D2, E2 and F2 had body score ranged from 7.93 to 8.11. The lowest for body was reported for culture G2 and H2.

Color and Overall acceptability: Lowest score was observed in culture A2 (7.97). Cultures A2, B2, C2, D2 and H2 shown non-significant difference ($p < 0.05$), but they were significantly lower than rest of the cultures. Cultures control, C2, D2, F2, G2 and I2 were at par. Culture E2 showed best color and appearance profile than rest of the cultures. Highest overall acceptability score for culture J2 (8.350) and lowest for culture H2 (7.52) and the average score 7.89. Cultures control, I2 and J2 showed higher value and were at par. Culture C2, E2 and F2 showed overall score 7.84, 8 and 7.95 respectively, they were at par.

CONCLUSION

Dahi and its related products were, are and will remain an essential part of the daily diet of Indian population. This study shows that the final four cultures (I2, E2, J2, F2) have relatively good physicochemical and sensory characteristics and can be used as a standard culture for *Dahi* making. Among these four cultures higher overall sensory score was observed for cultures I2 and J2. It is concluded that the better quality *Dahi* can be prepared by using cultures I2 and J2.

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Flavour							
Body							
Acidity							
Colour and appearance							
Overall Acceptability							

Preference: > > >

Comments:

Name:

Date:

Signature