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ANTIOXIDANT AND QUALITY ATTRIBUTES OF *DAHI* FORMULATED USING POTENTIAL PROBIOTIC STRAIN ISOLATED FROM INFANT FECAL

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ABSTRACT

Objective of the present study was to formulate dahi using potential probiotic strains isolated from infant fecal as well as to study their antioxidant, physicochemical, microbiological, and sensory properties. Three lactic acid producing species were isolated from infant fecal. Three different dahi samples (EX-I, EX-II and EX-III) were prepared using isolated strains. The EX-IV dahi was prepared using combination of isolate II and III. From the samples pH, acidity(%), synersis(%), and total solids(%) were estimated and the range were 3.83-5.00, 1.25–2.04, 22.33–29, and 12.40–18.00%, respectively. The total phenol was highest (11.39 mgGAE/100g) in EX-I dhai. The total antioxidant capacity (FRAP & ABTSRSA) was found higher in EX-IV compared to all other samples. The LAB count was highest in EX-II and EX-IV (≤9 log cfu/g). The highest sensory score was obtained for control followed by EX-I. In conclusion, the isolated potential probiotic strains could be used for functional food product development.

Keywords: isolated probiotic strain, dahi, antioxidant, microbial

INTRODUCTION

Dahi is an indigenous Indian fermented milk product known for its stimulating taste, palatability and curative values; it also called as 'curd' **[1]**. *Dahi* is a popular fermented milk product in India consumed in almost every household **[2]** by all age groups from infancy to geriatrics. It is prepared from buffalo milk, cow milk or standardized milk **[2]**. As per PFA rules**, "***dahi* or curd is a product obtained from pasteurized or boiled milk fermented with a culture". The different starter culture used in the manufacture of commercial *dahi* namely *Lactococcus. lactis, Streptococcus thermophilus, Lactobacillus bulgaricus, Lactobacillus. Plantarum, Lactobaccilus. cremoris,* and lactose fermenting yeasts **[3]**. Around 90% of the total fermented milk products produced in India is in the form of *dahi* **[2]**. Among the various cultured milk products, the international market for *dahi*/curd and chilled desserts has been benefited due to extensive growth toward healthy food and due to new product development **[2]**. From the last decade demand for *dahi*/curd has increased greatly, because of it's nutritional and therapeutic benefits **[2]**.

Although in the traditional practice lactic acid bacteria (LAB) involves as a starter cultures for milk fermentation, they can also be serve as protective cultures against microbial pathogens

and spoilage organisms in minimally processed foods **[4]**. Some lactic acid bacteria act as important probiotic because of their strain-specific properties that are beneficial to health **[5]**. To action as probiotics, bacterial strains should meet certain requirements such as tolerance to high acid and bile environment **[6]** and many more. Fermented milk products with probiotic organisms have gain special attention due to their confirmed health claims**[7].**

In recent time, natural antioxidants from natural sources have gain popularity over synthetic one. Protein hydrolysates and peptides from different plant and animal protein showed good antioxidant and ACE inhibition capacity**[7].** Various studies reported a proteolytic process during fermentation of milk by lactic acid producing bacteria. Thus, the need of probiotics with antioxidant activity to minimize oxidative stress is a new alternate to maintain a good health**.**

The ecosystem of gastrointestinal microflora is a very complex [**8]**. within the intestinal microflora more than 400 species identified and many of them population levels nearly as high as 10^{12} per g in the colon [**7]**. Initial colonization of the aseptic intestine of the newborn happens during birth when bacteria from the mother's intestinal and vaginal flora inoculate the gastro-intestinal tract of the fetus **[9].** Lactobacillus strains are found naturally in the human intestine; therefore, such strains are specially developed for commercial use as probiotics [**10**]. Some researchers reported that bacteria isolated especially from the feces of infants or elderly humans possess potential probiotic properties [**10**]. Now days, probiotics are known as potential, novel and natural therapeutic drugs [**11**]. Thus, the isolation and characterization of new strains are still needed [**12]**.

Thus, the aim of this study was to isolate lactic acid strain from infant fecal sample and formulation of *dahi* using isolated lactic acid strain as well as to evaluate physicochemical, antioxidant, microbiological, and sensory properties of *Dahi* compared to market dahi.

METHODOLOGY

A. Isolation of strains

An eight-month-old infant (healthy and without any prenatal problems) was a donor. The fecal sample was collected in a sterile container. Five gram of the feces was suspended and homogenized in 45 ml of phosphate buffered saline (PBS) pH 7.4. The homogenized feces were used to prepare the 10-fold serial dilutions. 0.1ml of an appropriate dilution were spread-plated on Man, Rogosa and Sharpe agar (MRS) and incubated at 37°C under anaerobic conditions in an anaerobic jar. After 48 hour of incubation, three colony was based on size selected among them two were big and one was small in size. All lactic acid bacterial isolate were inoculated in MRS broth at 37°C for 24 hour. The culture stock was prepared in 50% glycerol and stored at -20°C until use.

To prepare the starter culture, the broth culture was centrifuged at 8000 rpm for 15 min, and the

pellet was washed twice with 0.9% NaCl and 1ml of washed culture were suspended in sterile skim milk tubes. These tubes were incubated overnight at 37°C, subsequently in the skim milk successive five transfers of the cultures were given before used as a starter culture for *dahi* preparation

B. Formulation of *Dahi*

400ml of UHT treated toned milk was purchase from Amul outlet (Anand). Milk was heated till obtained 45°C. Milk was divided into four equal portions (100ml x 4) and in the aseptic condition 2% of activated cultures of isolated strain were added into each 100ml of milk. The samples were incubated at 42°C until the coagulation completed (17 to 22 hrs). Cooled the samples at 4°C for 4hr. Four samples were prepared using 3 isolated strains. Three experimental *dahi* samples were cultured using three different isolates (i.e EX-I,EX-II and EX-III) and fourth *dahi* sample was cultured using combination of isolate II & III (EX-IV). Market *dahi* was used as control sample.

C. Parameter studied

Experiments were carried out in duplicate. *Dahi* samples were collected and subjected to physicochemical, Antioxidant, microbiological, and sensory properties.

i. Physicochemical Properties

pH was measured using a pH meter (Systonic) after pH calibration with standardized solutions to pH 4 and 7. Titratable acidity in % lactic acid was measured according to **BIS [13]** method. The syneresis of *dahi* samples was determined as described by **Prasanna et al. [14]**. The total solids and moisture content of all the samples was determined according to the Association of Official Analytical Chemists method **[14]**.

ii. Antioxidant Properties

Total phenol content was measured with Folin-Ciocalteu's phenol reagent using the method described by Cho et al. [**16**]. For comparison gallic acid was used as a standard and the results are expressed in mg GAE/100 g.

Total antioxidant capacity of the *dahi* samples were determined by using ferric reducing antioxidant power assay (FRAP) [**17**] where trolox was used as standard for the comparison and results are expressed as mg TE/100 g while DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and ABTS [2, 2 Azinobis (3–ethylbenzothiazolin-6-sulfonic acid) diammonium salt] radicals scavenging activity were determine as described by Brand-Williams et al. [**18**] and Re et al. [**19**], respectively where percentage inhibition was calculated and results are expressed as mg TE/100 g sample.

iii. Microbiological Assay

10 g of the *dahi* samples was collected aseptically and blended using a stomacher (Minimix 100Pc) with 90 ml of sterile 0.1 % peptone water then submitted to serial dilutions with the same diluent. The count of isolated probiotic strain was estimated by spreading 0.1 ml of each dilution, respectively in petri-dish

containing DeMan-Rogosa-Sharpe agar (MRS agar). Yeast and mold, *E. coli* and total coliform population were enumerated on Potato Dextrose agar, Hi-chrome agar and violate red bile agar (VRBA), respectively. Petri plates were incubated at 37 °C for 48 h. At the end of incubation colonies were counted and the results expressed in log cfu/g.

iv. Sensory Evaluation:

The acceptability of the experimental and control *dhai* was assessed by the modified hedonic score card where a panel of six judges tasted and scored the product on a scale of 1-9, where 1 = extremely dislike and 9 = extremely like for the sensory attributes of odor, texture, syneresis, acidity, taste and flavor as well as for overall acceptability .

D. Statistical analysis:

All the data were expressed as Mean ± SD. To analyze the results, one-way analysis of variance (ANOVA) and Duncan test ($p \leq 0.05$) were used. Statistical analysis was performed using the SPSS 20 version.

RESULTS AND DISCUSSION

Among the selected three isolates on MRS agar, morphologically two big size colonies were rod shape and one small size colony was cocci, all were gram positivity, and catalase negativity, all isolates were identified as lactic acid bacteria through biochemical test. These strains were also analyzed for probiotic potential and results showed that all three strains were tolerated pH3 and 1% bile. The data was not discuses in this paper.

i. Physicochemical Properties

The pH of control and experimental samples ranged between 3.88 to 5.00 (table 1) where EX-IV showed significantly ($p \leq 0.05$) lower pH and EX-II showed significantly($p \leq 0.05$) higher value compared to control and EX-I and EX-III *dahi* samples. The optimum pH of market thick fermented milk is from 3.27 to 4.59 [**15]**, in the present study pH of *dahi* samples also fell into this optimum range except pH of EX-II *dahi*. Similar range of pH also reported by **Rasdhari et al. [20], Patel et al. [21]**, **Sudhir et al. [3]** and **Cho et al. [16] in** their studies.

Values of acidity (%) were observed inversely proportional to the pH value of *dahi* samples. Control and experimental *dahi* samples acidity were ranged from 1.25 to 2.51 % (table 1) where EX-IV showed significantly($p \leq 0.05$) higher acidity and EX-II and EX-III showed significantly($p \leq 0.05$) lower acidity. Similar range of acidity also reported by **Rasdhari et al. [20], Sudhir et al. [3] and Cho et al. [16]** in their studies i.e. 0.81 to 0.83%, 0.52 to 1.26 and 0.92 to 0.94%, respectively.

The synersis of all experimental *dahi* samples except EX-II showed significantly($p \leq 0.05$) lower values (22.33 to 26.00%) compared to control *dahi* (29.00%). According to **Fox et al., [22]** Syneresis is directly affected by acidity and is inversely proportional to pH. This statement was also true for this study. Synersis (%) content of control and experimental samples ranged between 18.25 to 22.5 % by **Rasdhari et al. [20]**

and 14.28 to 25.55 by **Cho et al. [16].** In the present study similar values were observed for sample EX-I, EX-III and EX-IV while control and EX-II showed slight higher synersis (%).

Total solids (%) and moisture (%)content of control and Experimental *dahi* samples were ranged from 12.40 to 18.00 and 82.00 to 87.60(%), respectively (table 1), where control *dahi* showed significantly ($p \leq 0.05$) higher total solids content and EX-II showed significantly ($p \leq 0.05$) lower total solids content among all the *dahi* samples. Results of moisture content were inversely proportional to results of total solids content. Similar range of total solids content was also observed by **Rasdhari et al. [20]** and **Sudhir et al. [3]** i.e 24.85 to 25.47% and 23.87 to 36.37%, respectively.

Among all the experimental *dahi* samples isolates I (EX-I) and combination of isolate-II and III (EX-IV) took less time (i.e 17hr) for coagulation while when isolate –II and III add singly it took higher time (i.e 22hr) for coagulation. They also shown a lower acidity compared to combination (i.e EX-IV).

ii. Antioxidant Properties

Probiotic organisms act as an antioxidant by changing the redox status of the host by metal chelating, antioxidant capacity, regulating signaling pathways, inhibiting enzymes producing ROS etc. [**23**] while total phenolic content (TPC) present in milk may be due to the presence of polyphenols in milk, that mostly come from the feed **[24]** as well as because of the protein and reducing compounds **[25]**. The various microbiological cultures degrade the such primary components of milk such as protein, carbohydrate and lipids into various secondary forms like free amino acids, peptides, organic acids, free fatty acids, where the free amino acids and peptide sequences owns antihypertensive effect **[26]**, increasing of antioxidant capacity and inhibition of lipid peroxidation [**27]** this functions can be very beneficial for the human health and wellbeing. It is reported that various strains of lactobacillus showed antioxidant capacity in the yoghurt [**23**]. Hence in this study antioxidant capacity and total phenol were measured.

Mean values of total phenol content, and total antioxidant activity measured using FRAP, DPPH and ABTS can be seen in Table 2.

The mean values of total phenolic content equivalent to gallic acid of control and experimental *dahi* samples ranged between 4.53 to 11.39 mg/100 g (table 2) where control *dahi* showed significantly ($p \leq 0.05$) lower total phenol content compared to all experimental samples, Ex-I *dahi* showed significantly ($p \leq 0.05$) higher (11.39 mg/100g) total phenol content followed by EX-III *dahi* sample (10.29 mg/100g). Similar result was reported by **Cho et al. [16]** i.e 4.3 to 6.9 mg GAE/100g while **Sudhir [3]** and Dabija et al. **[7**] reported higher

Total phenol content, 218 mg/100g and 0.99 GAE/g, respectively compared to results of the present study.

FRAP assay has been reported to be suitable to measure antioxidant activity of substances having half-reaction redox potential below 0.7 V. This measures only non-protein antioxidant capacity. Milk component such as urate, ascorbate, alpha-tocopherol and bilirubin have been characterized to have ferric reducing ability [**28**]. The total antioxidant capacity estimated by FRAP assay measures the effect of antioxidant of any substance in the reaction medium as reducing ability of the complex Fe(III)-TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) [**29]**. Ferric reducing antioxidant power of control and experimental samples ranged between 27.50 to 36.32 mg TE/100 g (table 2), where EX- IV followed by EX-I *dahi* sample showed significantly ($p \leq 0.05$) higher value (36.32 and 34.72, respectively) compared to EX-III (27.5 mg TE/100 g) *dahi*. Compared to the present study **Sudhir [3]** reported lower FRAP content (111 µM trolox/g) while **Chouchouli et al.[25]** reported higher FRAP content (96mg AAE/100g) in their study.

The DPPHRSA assay is a simplest method which gives information on the radical scavenging activity of the antioxidant substances present in a sample **[28]**. The mean value of DPPHRSA of control and experimental *dahi* samples ranged between 2.64 to 7.09 mg TE/100 g (Table 2) where control *dahi* showed significantly ($p \leq 0.05$) higher (7.09 mg TE/100g) value compared to all experimental *dahi* samples. Similar range of DPPHRSA also reported by **Sudhir et al. [3] and cho et al. [16]** which were 53 µM trolox/g and 42 to 47% inhibition, respectively. While **Dabija et al. [7]** obtained lower DPPHRSA (10.56% inhibition) compared to present study.

The antioxidant capacity of foods can be measured using autobleaching of a preformed solution of ABTS radical cation **[29]**. The decolourization of the solution takes place in the presence of hydrogen donating antioxidant (nitrogen atom quenches the hydrogen atom) [**29].** The ABTS radical scavenging activity was found in the range of 3.52 to 5.48 mg TE/100 g (Table 2) where EX-II *dahi* showed significantly ($p \leq 0.05$) higher (5.84 mg TE/100g) value and control *dahi* showed significantly($p \leq 0.05$) lower (3.52 mg TE/ 100g) value compared EX-I, EX-III and EX-IV *dahi* sample. Compared to the present study **Sudhir et al. [3]** reported higher ABTSRSA (245µM trolox/g).

iii. Microbial Analysis

Microbiological characteristics are indicators of quality, safety and shelf-life of the prepared product. For the microbial analysis of *dhai* samples, probiotic lactic acid bacterial were enumerates on MRS agar and contamination bacteria such as *E.coli*, total coliforms and yeast and mold enumerated on *E.coli* Hi-crome agar, Violate red bile agar and potato dextrose agar, respectively.

The mean value of probiotic lactic acid bacterial count was ranged between 6.05 to 9.20 log cfu/g (Fig;1) where control *dahi* sample showed zero count. Among experimental sample EX-I had significantly ($p \leq 0.05$) lower count compared to EX-II and EX-IV. The highest count was found in EX-IV. No growth in control *dahi* could possible due to commercial cultures as this *dahi* sample is commercially produced. FAO/WHO recommendation that if food sold with health claims from the addition of probiotics it must contain, per gram, at least 10^6 to 10^7 cfu/ml of viable probiotic bacteria **[30]** and in the present study all experimental samples fulfill this criterion. Various researchers reported lactobacillus count 7.00 to 9.38 log cfu/ml in the fermented milk (**[16], [3] and [20]**).

Contamination of *E.coli*, total coliforms and yeast and mold were observed in any experimental samples while control sample showed contamination of *E.coli* and total coliforms (3.21 and 3.10 log cfu/g) (Plate:1). Yeast and mold contamination was not found in any of the *dahi* samples .

iv. Sensory evaluation

Sensory evaluation of dahi sample was carried out using modified hedonic card for the different sensory attributes such as odor, texture, synersis, acidity, taste and flavor as well as for over all acceptability. Only two experimental samples were analyzed for sensory attributes i.e. EX-I and EX-IV, which take lower coagulation time (i.e 17hr).

The mean score of odour, texture and synersis were ranged between 6.20 to 7.40 and statistical analysis showed no significant difference between control and experimental *dahi* samples (Fig:2). The value of the pH and acidity was effect the sensory perception of *dahi* samples. The acidity values EX-IV *dahi* sample was higher which affect on the sensory perception and this sample showed significantly lower score for acidity, taste and flavor as well as for overall acceptability(OA) (5.6,6.0, and 6.2, respectively). Control sample obtained higher score for all the sensory attributes (7.2 to 8) followed by EX-I (6.8 to 7.2) *dahi*

CONCLUSION

From the results obtained it is concluded that the isolated strains posses good antioxidant properties and formulated a good quality curd. Hence, they could be further used as a potential probiotic culture to produce milk fermented products and also to prepare non- dairy fermented foods or synbiotic foods.

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Table 1: Physicochemical properties of control and experimental *dahi.*

Samples	pH	Acidity (%)	Synersis (%)	Total solids (%)	Moisture (%)	Coagulation time (hr.)
C	4.22±0.00^{b,c}	1.45±0.01^{b}	29.00±0.00^{c}	18.00±0.00^{d}	82.00±0.00^{a}	--
Ex-I	4.00±0.00^{b}	2.04±0.05^{c}	22.33±0.58^{a}	13.40±0.00^{b}	86.60±0.00^{d}	17
Ex-II	5.00±0.00^{d}	1.25±0.02^{a}	28.67±0.58^{c}	12.40±0.00^{a}	87.60±0.00^{d}	22
Ex-III	4.66±0.00^{c}	1.34±0.02^{a}	26.00±0.00^{b}	13.00±0.00^{b}	87.00±0.00^{c}	22
Ex-IV	3.83±0.00^{a}	2.51±0.11^{d}	22.33±0.58^{a}	14.67±0.58^{c}	85.33±0.58^{b}	17
F- value	264.2*	256.47*	159.167*	253.36*	253.36*	--

Mean ±SD of three trials
Means carrying different superscripts within a column are significantly different at $p \leq 0.05$
*indicate significant difference ($p \leq 0.05$)

Table 2: Total phenol content and total antioxidant capacity of control and experimental *dahi.*.

Samples	Total Phenol (mg GAE/100 g))	Total antioxidant capacity (mg TE/100 g)		
		FRAP	DPPHRSA	ABTSRSA
C	4.53±0.23^{a}	31.53±0.97^{a,b}	7.09±2.86^{b}	3.52±0.12^{a}
Ex-I	11.39±0.29^{d}	34.72±4.17^{b}	3.34±0.61^{a}	4.88±0.96^{a,b}
Ex-II	7.55±0.07^{b}	32.01±1.87^{a,b}	3.72±0.83^{a}	5.84±1.55^{b}
Ex-III	10.29±0.15^{c}	27.50±1.39^{a}	2.64±0.77^{a}	5.01±1.04^{a,b}
Ex-IV	7.40±0.39^{b}	36.32±3.4^{b}	4.36±1.16^{a}	5.70±0.80^{a,b}
F- Value	340.62*	4.85*	3.95*	2.08

Mean ±SD of three trials
Means carrying different superscripts within a column are significantly different at $p \leq 0.05$
*indicate significant difference ($p<0.05$)

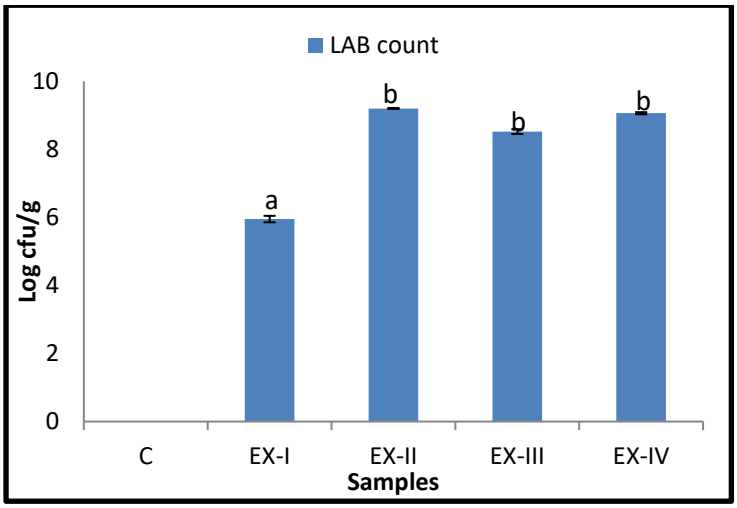

Fig.1: LAB count (log cfu/g) of control and experimental *dahi.*
Mean ±SD of three trials
Means carrying different superscripts are significantly different at $p \leq 0.05$

Plate:1 *E.coli* and total coliforms count on Hicrome *E.coli* agar and VRBA petriplate, respectively.

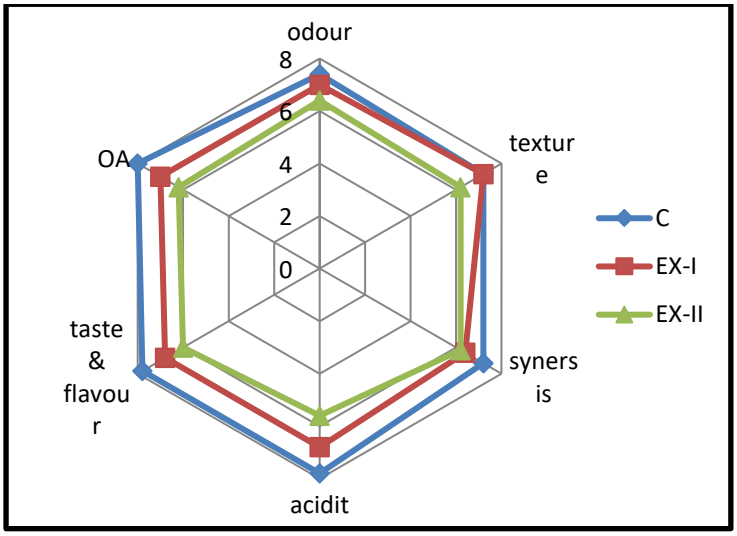

Fig:2 Sensory attributes score of control and experimental *dahi.*