Process Standardization for the Manufacture of *Shrikhand* Spread

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Abstract: *Purpose*: *Shrikhand* Spread, a unique sweetened fermented Indian milk product is made by separation of whey from *dahi*, the Indian counterpart of Western yoghurt, followed by addition of sugar. Production of *dahi* employing traditional method involved undefined mixed starter cultures, uncontrolled fermentation and longer production time resulting in wide variation in its chemical and microbiological qualities. In order to cater to a product with desirable properties like lower post-acidification, higher flavour profile, firm body and lower syneresis coupled with shorter production time, conjugated application of yoghurt cultures and *dahi* cultures were tried. Since the shelf-life of *dahi* is limited, conversion into *shrikhand* spread may be used as a tool to extend the shelf-life and therefore the market reach towards commercialization into the global market as a potential functional food.

Design/Methodology/Approach: Different batches of *dahi* were made from homogenized (Stage I - 2500 psi, Stage II - 500 psi) and pasteurized (74-78 °C/16-19 Sec) milk, pre-adjusted to 3.15-3.20% fat and 11.40% snf with diverse starter combinations selected upon the extent of post acidification, volatile acid production, syneresis and rheological characteristics. Homogenized, pasteurized and regulated milk was further subjected to a heat-treatment (90°C/10 min) and seeded with selected starter combinations to obtain firm curd intended for *shrikhand* spread manufacture. Shelf-life of *shrikhand* spread was evaluated in terms of chemical and microbiological criteria upto 7 days of storage at 8±1°C.

Findings: Starter combination of eXactDahi 2+YoFlex Express 1.0 at an incubation temperature-time combination of 45°C/5h was found most suitable for producing *dahi* with smooth body, higher volatile acidity and low syneresis. Utilization of *dahi* obtained employing the above starter combination for the manufacture *shrikhand* spread was suggested and the product was found to retain its goodness when stored for 7 days at 8±1°C.

Originality/Value: Conjugated use of yoghurt cultures with *dahi* cultures was suggested to overcome the drawbacks of traditional process of *dahi* manufacture suitable for conversion into *shrikhand* spread. This *dahi* was found capable of enhancing its dietetic value in addition.

Keywords: Lactic acid bacteria *Dahi*, *Chakka, Shrikhand* spread, Shelf-life.

INTRODUCTION

Elaboration of biologically active peptides exhibiting both functional and physiological roles *in vitro* and *in vivo* by lactic acid bacteria (LAB) have been drawing serious interest for its applications in food and nutrition science [1,2] resulting in consumer's inclination towards healthful foods [3,4].

The three major Indian fermented milk products namely *dahi* (curd), *shrikhand* (sweetened concentrated curd) and *lassi* (stirred curd), might be considered as the Western counterpart to yoghurt, quarg and stirred yoghurt, respectively. Nutritional and therapeutic significance of *dahi* [5,6] and *shrikhand* [7-9] is well documented. Reviewed literature indicated that functional properties of traditional *dahi* could be enhanced either by manipulation of fortifying ingredients of basic mix, starter combinations and incubation temperature and time coupled with the adoption of appropriate packaging material [6] and their subsequent use for the manufacture of *shrikhand* spread would help towards process standardization and project them into the global market as a functional food.

Traditional method of *dahi* production involving natural culturing of boiled cow, buffalo or mixed milk with undefined starter cultures and uncontrolled long fermentation could not be practical for industrial production. Conjugation application of yoghurt cultures capable of elaborating exopolysaccharides (ESP) along with normal *dahi* cultures might result in more acceptable product in terms of body, texture and flavour and greater antibacterial activity against pathogenic organisms. Microstructural study showed that *dahi* made with EPS-producing strains had relatively compact linear structure with more open structure and pores having discontinuous casein matrix than the controlled *dahi* [10].

Shelf-life of *dahi* is longer than milk but it is still limited. *Shrikhand* is known for its extended shelf-life mainly due to its higher acidity & reduced water content [11,12] and change in osmotic concentration due to addition of sugar [13] and therefore have greater market reach. Traditionally made *shrikhand* has very high total solids and sugar content and is not preferred by the health conscious consumers of present

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generation. In the present study an endeavor has been made to develop a proprietary fermented dairy product named as *shrikhand* spread to reciprocate the consumer's demand for a *shrikhand* variety with lower total solids and sugar. Compensation of comparatively lower fat, protein and total solid in *shrikhand* spread has been conceptualized in the current study by application of ESP producing starter cultures. Yoghurt cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*) were capable of producing ESP [14] but they failed to produce diacetyl [15], whereas *dahi* cultures had characteristic diacetyl flavour [16], therefore in the present investigation attempts were made to obtain *dahi* employing mixed cultures of *dahi* and yoghurt cultures and their subsequent utilization for the production of *shrikhand* spread with diacetyl flavor and enhanced shelf-life.

MATERIALS AND METHODS

Type of Milk

Raw mixed milk received from Nadia and Murshibad districts of West Bengal, India was standardized to 3.15-3.20% fat and 11.40% snf by addition of BIS standard skim milk powder and white butter to obtain *dahi* intended for *shrikhand* spread manufacture.

Starter Cultures

Freeze dried cultures of Yoghurt NCDC-144, Dahi NCDC-167 and Dahi NCDC-159 (obtained from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal, India) and direct to-vat inoculation cultures of JOINTEC VB529, STA IDC701 (obtained from Centro Sperimentale del Latte S.r.l., Italy), Yo Flex Express 1.0, eXactDahi 2, CHN-11, STI-13 (obtained from Chr Hansen, USA) and YO-MIX 336, LYO DAHI (obtained from Danisco France SAS, France) were applied in diverse combinations to obtain *dahi*. Freeze dried cultures were activated after three consecutive transfers and maintained in sterile skim milk.

Selection of Starter Cultures for *Dahi* Manufacture

Homogenized (Stage I - 2500 psi, Stage II - 500 psi) and pasteurized (74-78 °C/16-19 Sec) milk standardized to 3.15-3.20% fat and 11.40% snf was seeded with diverse starter cultures to obtain *dahi*. Based upon extent of post acidification, volatile acid production, syneresis and rheological characteristics, best quality *dahi* intended for *shrikhand* spread manufacture was selected.

Preparation of *Dahi* and *Shrikhand Spread*:

Homogenized and pasteurized standardized milk was subjected to the suggested heat-treatment of 90°C/10 min [17], followed by cooling to 30 to 45°C for inoculation with selected starter combinations. Inoculated milk was then incubated for 4 to 5h to obtain firm curd intended for *shrikhand* spread manufacture.

Dahi obtained by the above method was strained for 12h under refrigerated condition (5 ± 2°C) through polyethylene filter bags, pre-sterilized using 0.1% Suma Bac (Sealed Air) to obtain *chakka*. *Chakka* was then admixture with 40% boiled sugar syrup to obtain *shrikhand* spread. Shelf-life study of *shrikhand* spread was carried at 8±1°C/7days. Flow diagram for the production of *shrikhand* spread shown in Figure **1**.

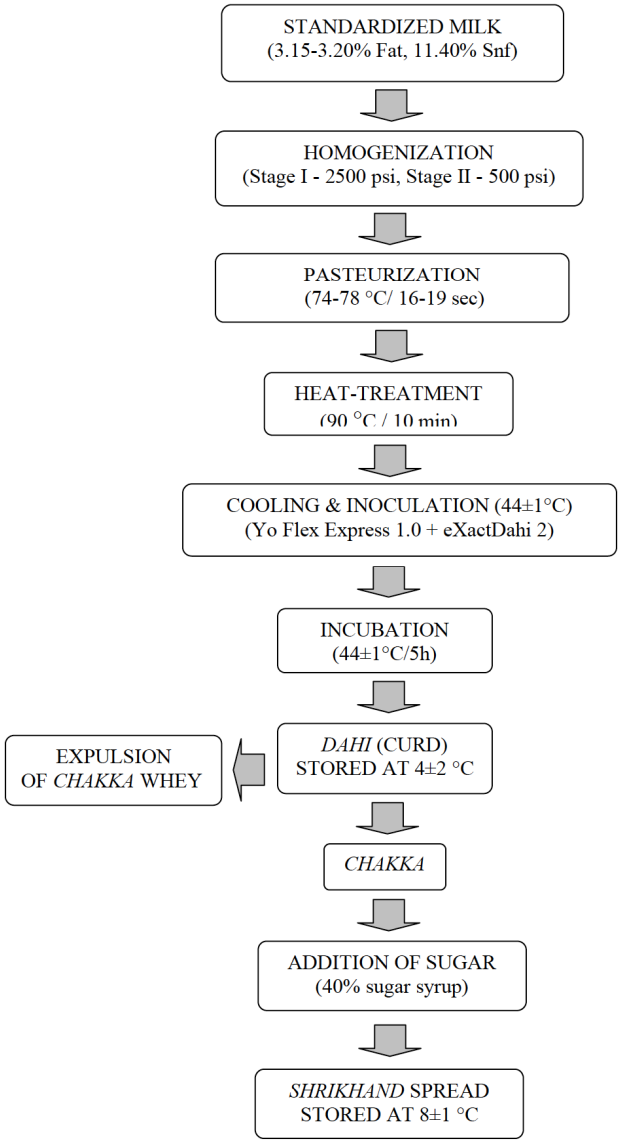

Figure 1: Method of production of *shrikhand* spread.

Analytical Techniques

Chemical Attributes

Raw milk free from different preservative and adulteration as prescribed in the methods delineated in laboratory manual [18] was standardized for *dahi* manufacture. Titratable acidity, % Fat and % snf of standardized milk was determined by the methods of [19].

Dahi, *chakka* and *shrikhand* spread was analysed for fat, ash content, titratable acidity [20, 21], volatile acidity [22], total solids using Moisture Analyzer-HB43-S (Mettler Toledo, Switzerland) and protein using 2006 Digestor and Kjeltec System 1002 Distilling Unit (Foss, Denmark). Syneresis of *dahi* was determined by centrifuging 50 ml of *dahi* at 2600 rpm at 8ºC for 10 min and expressed as a weight percentage of the whey separated from the gel over the initial weight of the gel [23].

Microbiological attributes

Microbiological attributes of milk, *dahi*, *chakka* and *shrikhand* spread were evaluated on the basis of total viable microbial contents using nutrient agar (Hi-Media, Bombay, India), coliform count, *Escherichia coli*, *Salmonella* Count using MacConkey agar (Merck, Germany), *Listeria monocytogens* using autoclaved Polymixin Acriflavine Lithium Chloride Ceftazidime Esculin Mannitol (PALCAM) broth and agar (Merck, Germany), yeast and mould using potato dextrose agar (Hi-Media, Bombay, India) adopting the methods of Bureau of Indian standards [19].

RESULTS AND DISCUSSIONS

SELECTION OF STARTER COMBINATIONS FOR *DAHI* MANUFACTURE

Yoghurt NCDC-144 produced lowest level of acidity (0.46 % lactic acid), greater value was noted, when grown in association with STI-13 (0.62% lactic acid) but the resultant curd had very soft body (Table **1**). Increase in acid production by STA IDC 701 was noted with the elevation of incubation temperature (37 to 42°C) but resulted in curd with bitter or salty taste. [24] reported increase in titratable acidity, excessive proteolysis and development of bitterness in yoghurt, which is influenced by species and strain of starter culture used. Prolonged incubation time (4 to 5 h) at 42°C induced a retarded acid development in *dahi* accompanied with lower volatile acidity (0.72 ml of 0.1NNaOH/ 50g curd), higher syneresis (12.29 %) and salty taste, indicating inhibition of STA IDC 701 due to developed acidity. Beal *et al*. (1999) [25] observed that the incubation temperature influenced the microbial metabolism and the consequent lactic acid fermentation.

No significant effect in *dahi* obtained with JOINTEC VB530 was observed either with the increase in incubation temperature (37 to 45°C) or due to the conjugated use with STA IDC 701. JOINTEC VB530 was found not suitable for *dahi* manufacture due to development of ropiness accompanied by higher post acidification (1.04% lactic acid) after 3 days of storage at 8±1°C, which is beyond [20] specification. Acidity development by JOINTEC VB530 in association with STA IDC 701was within the prescribed limits of BIS (1980 a) [20] upto 7 days of storage (0.86-0.92% lactic acid) but had a lower flavour profile (0.22 ml of 0.1NNaOH/ 50g curd). Lower firmness in yoghurt made using EPS producing starter was observed than those made with non EPS producing starter cultures starter [26, 27] might be attributed to differences in the amount and molecular characteristics of EPS and their ability to interact with proteins [28].

Dahi made with LYO DAHI and YO-MIX 336 had higher volatile acidity than JOINTEC VB530 but showed higher syneresis (19.80-21.08%). Such behavior indicated lower or no production of ESP by cultures involved. Quantum of EPS produced by *S. thermophilus* was higher (50 to 350 vs. 60 to 150 mg/L) than those elaborated by *L. delbrueckii* subsp. *bulgaricus* [29,30], therefore total ESP production in *dahi* was dependent on the species of lactic acid present. Post-acidification (0.70 to 0.83% lactic acid) encountered in *dahi* made with either Dahi NCDC-167 or Dahi NCDC-159 was within BIS (1980 a) [20] standards but the resultant product was found with weaker body.

Dahi obtained with YoFlex Express 1.0 attained an acidity of 0.75% lactic acid with no whey separation and when grown in association with STI-13 resulted in *dahi* with grainy structure but without significant change in regards to acidification extent (0.78% lactic acid) even after 7 days of storage. An elevation in incubation temperature (42 to 44°C) or incubation period (5 to 5.50h) induced higher post-acidification (1.03 to 1.05% lactic acid) in *dahi* within 01 day of storage, which is beyond [20] specification. No significant difference in post-acidification of *dahi* made with eXact Dahi 2 (0.60 to 0.71% lactic acid) could be noted with the inclusion of CHN-11(0.59 to 0.74% lactic acid) during 7 days of

Table 1: Effect of Starter Combinations and Incubation Conditions on Quality of *dahi*

Starter Combinations	Incubation Conditions	Volatile Acidity (ml of 0.1NNaOH/ 50g curd)	Syneresis (%)	Titratable Acidity (% Lactic acid) After storage at 8±1°C (days)				Rheological Properties
				0	1	3	7	
Yoghurt NCDC-144	42°C/5h	-	-	0.46	-	-	-	Partial Curdling
Dahi NCDC-167	30°C/5h	-	-	0.70	0.72	0.80	0.83	Soft body
Dahi NCDC-159	30°C/5h	-	-	0.70	0.70	0.77	0.78	Soft body
JOINTEC VB530	37°C/5h	-	-	0.74	0.98	1.04	1.04	Ropiness noticed
	45°C/5h	0.45	2.04	0.75	0.96	0.92	1.00	Slightly soft body
STA IDC 701	37°C/5h	-	-	0.55	0.83	0.80	0.88	Bitter taste
	42°C/4h	-	-	0.68	0.88	0.85	0.90	Bitter taste
	42°C/5h	0.72	12.29	0.63	0.85	0.85	0.85	Slightly Salty
LYO DAHI	45°C/5h	0.89	21.08	0.59	0.83	-	-	Higher Syneresis
YO-MIX 336	45°C/5h	0.50	19.80	0.71	0.87	-	-	Higher Syneresis
YoFlex Express 1.0	42°C/4h	-	-	0.62	0.74	0.74	0.75	No whey separation
eXact Dahi 2	37°C/5h	0.62	-	0.60	0.70	0.70	0.71	Hard body, Grainy
STI-13	42°C/4h	-	-	0.67	0.72	0.74	0.77	Grainy body
Yoghurt NCDC-144 +STI-13	42°C/4h			0.62				Very Soft body
JOINTEC VB530 + STA IDC 701	42°C/4h	-	-	0.70	0.89	0.81	0.86	Slightly weak body
	42°C/5h	0.22	3.80	0.68	0.88	0.92	0.92	Slightly weak body
YoFlex Express 1.0+ STI-13	42°C/4h	-	-	0.67	0.78	0.78	0.78	Grainy texture
	44°C/5h	-	-	0.76	1.05	1.10	1.15	Grainy texture
	42°C/5.25h			0.79	1.03	-	-	Grainy texture
	42°C/5.50h			0.82	1.04	-	-	Grainy texture
eXact Dahi 2+ CHN-11	37°C/5h	0.73		0.59	0.71	0.72	0.74	Slightly weak body, No grains
STI-13+ CHN-11	37°C/5h	1.35	1.74	0.82	0.94	0.91	0.93	Soft body, Ropiness noticed
eXact Dahi2 + YoFlex Express 1.0+ STI-13	44°C/5h	-	-	0.83	1.02	1.03	1.03	Grainy texture
	42°C/5.25h	-	-	0.83	1.04	1.04	1.06	Grainy texture
	42°C/5.50h	-	-	0.83	1.01	1.04	1.04	Grainy texture
eXact Dahi2 + YoFlex Express 1.0	43°C/4h	-	-	0.63	0.68	0.72	0.72	Grainy body
	42°C/4h	0.80	0.10	0.84	0.91	0.91	0.93	Slightly Soft body, Ropiness noticed.
	42°C/5h	1.35	9.06	0.82	0.92	0.92	0.99	Slightly Soft body
	45°C/5h	1.40	2.23	0.88	0.93	0.93	1.00	Hard smooth body

storage. However, slightly higher values for volatile acidity (0.62 vs. 0.73 ml of 0.1NNaOH/ 50g curd) but slightly weaker body was noted in *dahi* due to associated growth of both the cultures.

Attempt was made to produce *dahi* with eXact Dahi2 + YoFlex Express 1.0 + STI-13 and to evaluate effect of diverse incubation temperature-time combinations on its quality. Irrespective of incubation temperature-time combinations adopted, all *dahi* samples had grainy texture and with higher developed acidity (1.01 to 1.04% lactic acid) after 1 day of storage, which is above [20] specification. Regardless of the incubation temperature-time combinations employed, conjugated application of eXact Dahi2 + YoFlex Express 1.0 resulted in *dahi* with low post-

acidification (0.72 to 1.00% lactic acid), which was within the prescribed limits of BIS (1980 a) [20]. Lower post-acidification in *dahi* obtained with eXact Dahi2 + YoFlex Express 1.0 indicated their capability to produce ESP. Both *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* have been reported to produce EPS [31] noted both EPS-producing as well as non-EPS-producing starter cultures utilized carbohydrates as energy source, whereas their carbon source was utilized for EPS formation by the former and for lactic acid and volatile production formation by the latter. An incubation temperature-time combination of 45°C/5h was found most suitable for producing *dahi* with hard smooth body, highest volatile acidity (1.40 ml of 0.1N NaOH/ 50g curd) and low syneresis (2.23%). Pourahmad and Assadi (2005) [32] also reported better sensory characteristics of yoghurt incubated at higher temperature (45°C) than those incubated at lower temperature (42°C). Lower syneresis in *dahi* encountered in the present investigation might be ascribed to ESP production by starter cultures. Behare *et al*. (2009) [33] reported capability of ESP producing lactic acid bacteria to bind significant amount of water. Higher incubation temperature (45°C) and longer incubation period (5h) was found more suitable to obtain *dahi* with higher flavouring compounds by *L. delbrueckii* subsp. *bulgaricus* and less chance of developing ropiness due to excessive polysaccharide production by *S. thermophilus*. During yoghurt manufacture higher incubation temperature and long incubation period caused an increasing preponderance of the rods [24], which are reported to produce less EPS than cocci [29,30].

Dahi made with JOINTEC VB530 was equally good as those made with eXact Dahi2 + YoFlex Express 1.0, but the former starter culture was not selected for *dahi* manufacture as the resultant curd had lower volatile acidity and slightly softer body, which might be attributed due to difference in characteristics of EPS elaborated by two cultures. Lower firmness in yoghurt made using EPS producing starter [26,27] might be attributed to differences in the amount and molecular characteristics of EPS and their ability to interact with proteins [28].

Chemical Quality of *Shrikhand* Spread

Chemical Composition

Chemical composition of *dahi*, *chakka* and *shrikhand* spread was analysed for contents of fat, total solids (TS), protein and ash (Table **2**). During conversion of milk to *dahi*, no significant difference in contents of fat, TS, protein and ash were noted regardless of the cultures used. Fat, total solids and ash content of *dahi* noted in the present investigation were lower than the reported values (3.98±0.14 to 4.25±0.12%, 25.49±0.45 to 36.03±1.11%, 0.87±0.02 to 0.96±0.00%, respectively) for skim milk *dahi* [34]. However, Goyal *et al.* (2016) [35] noted lower values for fat (2.96 to 3.12%), TS (11.36 to 11.78%) and protein (3.47 to 3.59) in *dahi* made from buffalo whole milk in contrast to those observed in the present investigation. Discrepancies in the chemical composition of *dahi* may be due to use of milk with different chemical attributes. It has been reported that the type of milk can influence the microbial metabolism resulting in final product with distinct chemical

Table 2: Effect of Starter Combinations on the Chemical Quality of *Shrikhand* Spread

Products	Fat (%)	Total Solids (%)	Protein (%)	Ash (%)	Volatile Acidity (ml 0.1N NaOH/50g curd)	Titratable Acidity (% Lactic acid)	Titratable Acidity (% Lactic acid)				
							After storage at 8±1°C (days)				
							1	3	5	7	10
Treated Milk	3.20	14.60	3.99	0.95	-	0.190	-	-	-	-	-
Dahi†	3.20	14.33	3.97	0.95	1.40	0.950	-	-	-	-	-
Chakka	8.16	24.55	10.40	0.88	1.50	1.45	-	-	-	-	-
Shrikhand	6.49	43.84	6.92	0.62	1.00	1.20	1.20	1.23	1.23	1.40	1.42
Treated Milk	3.15	14.50	4.02	0.93	-	0.165	-	-	-	-	-
Dahi††	3.15	14.16	3.97	0.92	0.45	0.990	-	-	-	-	-
Chakka	8.34	24.39	9.55	0.77	0.70	1.55	-	-	-	-	-
Shrikhand	6.12	36.95	7.91	0.68	0.70	1.23	1.24	1.27	1.27	1.52	1.60

†*Dahi* obtained by culturing milk inoculated with eXact Dahi 2+YoFlex Express 1.0 and incubated at 45°C/5h.
††*Dahi* obtained by culturing milk inoculated with JOINTEC VB530 and incubated at 45°C/5h.

composition [36] owing to their specific behaviour during the acidification process, which depends mainly on inherent characteristics of each milk source [37].

Regardless of the cultures used, during transformation of *dahi* into *chakka*, concentration of all chemical components except for ash content was recorded. An increment in fat, TS and protein content but a decline in ash content in *chakka* in relation to *dahi* indicated passage of mineral content of milk into *chakka* whey. Boghra *et al*. (1998) [38] noted higher total mineral content in buffalo milk *chakka* whey (0.65%) than in cow milk *chakka* whey (0.32%). Higher contents of protein (10.40 vs. 9.55 %) and ash (0.88 vs. 0.77%) noted in *chakka* made with eXactDahi 2+YoFlex Express 1.0 in contrast to those made with JOINTEC VB530, indicated greater retention of chemical ingredients probably due to binding nature of exopolysaccharide produced by former starter cultures. EPS production in milk by *L. delbrueckii* subsp. *bulgaricus* [39] and by *S. thermophilus* [40,41] was found capable of binding hydration water and interacting with other milk constituents such as proteins and micelles to strengthen the rigidity of the casein network [42].

Irrespective of culture type, *shrikhand* spread had lower contents of total solid and protein but fat and ash contents were within the prescribed limits of BIS (1980 b) [21] for *shrikhand*. Contents of fat, TS, protein and ash in *shrikhand* spread encountered in the present investigation were lower than reported values (12.07±0.26, 46.84±0.10, 7.27±0.28 and 0.82±0.02, respectively) for cow milk *shrikhand* [44] observed an increase in TS (67.96 to 68.14%) but a decline in contents of fat (8.75 to 8.68%) and protein (5.80 to 5.72%) during storage of *shrikhand* at 7°C for 21 days. Difference in composition of *shrikhand* might be attributed to the use of different type of milk, starter cultures involved and manufacturing techniques adopted [7].

Volatile Acidity

Flavour profile of *shrikhand* spread as determined by volatile acidity (ml of 0.1N NaOH/50g curd) indicated higher values for *dahi*, *chakka* and *shrikhand* spread made with eXactDahi 2 + YoFlex Express 1.0 (1.40, 1.50,1.00 ml/50g, respectively) than using JOINTEC VB530 (0.45, 0.70, 0.70 ml/50g, respectively). Vijayendra and Gupta (2014) [45] reported significantly higher volatile acidity for buffalo milk *dahi* (32.5 ml/50g) or cow milk *dahi* (29.2 ml/50g) in contrast to those encountered in the present investigation for both types of *dahi*. Reasons attributed to these discrepancies may be composition of milk or type of culture employed as synthesis of carbonyl compounds in yoghurt was influenced by the strains of yoghurt cultures involved [46,47]. Lower volatile acidity in spread obtained employing JOINTEC VB530 may be due to inhibition of flavor producing organisms as result of higher retention of lactic acid bacteria tending to higher acid development (1.23 % lactic acid). Production of higher level of volatile acidity by *L. delbrueckii* subsp. *bulgaricus* (2.0 ml/50g) than *S. thermophilus* (0.6 ml/50g) in pure culture [48] and a decline in microbial count of *L. delbrueckii* subsp*. bulgaricus* ($3.50x10^{10}$ to $2.9x10^{9}$cfu/ml), with an increase in acidity (0.58 to 0.66 % lactic acid) during 8 days storage at 16-24ºC [49] was reported.

Titratable Acidity

Enhancement in titratable acidity (0.190 to 0.950 % lactic acid) was noted during conversion of milk to *dahi* employing eXactDahi 2 + YoFlex Express 1.0, which further got concentrated (1.44 % lactic acid) in *chakka*. Higher increase in titratable acidity was noted in *dahi* (0.990 % lactic acid) and *chakka* (1.55 % lactic acid) while employing JOINTEC VB530 (Table **2**). Extent of acidification by *dahi* cultures *Lactococcus lactis* ssp. *lactis* C10, *Lactococcus lactis* ssp. *cremoris* C1, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* DRC10 at 30°C in buffalo skim milk sterilized at 121°C/15 min was reported as 0.93±0.2% lactic acid after 18h [50] whereas 0.96% lactic acid in standardized buffalo milk heated at 90°C/15 min after 14h [51]. Para *et al*. (2014) [43] recorded an acidity level of 0.83±0.02 % lactic acid while culturing boiled standardized milk (4% fat and 8.5% snf) with market sample of *dahi* at 25-28°C/12-14 h. Present investigation indicated that the type of milk, heat treatment, type of culture, incubation temperature and incubation period has a significant effect on the acidification process of *dahi*.

Shrikhand spread obtained using eXactDahi 2 + YoFlex Express 1.0 had a titratable acidity of 1.20 % lactic acid which gradually increased and attained the highest value (1.40 % lactic acid) after 7 days of storage. On the other hand *shrikhand* spread obtained employing JOINTEC VB530 had an acidity of 1.23 % lactic acid, which remained within the prescribed limits of 1.40% lactic acid [21] up to only 5 days of storage (1.27 % lactic acid). Results indicated greater metabolic activity of lactic acid bacteria flora of

Table 3: Effect of Starter Combinations on the Microbiological Quality of *Shrikhand* Spread

Products	After storage at 8±1°C (days)											
	Coliform (cfu/ml)				Yeast and Mould (cfu/ml)				*Staphylococcus aureus* (cfu/ml)			
	1	3	5	7	1	3	5	7	1	3	5	7
Treated Milk	Nil	-	-	-	-	-	-	-	-	-	-	-
Dahi†	Nil	-	-	-	Nil	-	-	-	-	-	-	-
Chakka	Nil	-	-	-	Nil	-	-	-	Nil	-	-	-
Shrikhand	Nil	Nil	Nil	Nil	Nil	14	25	32	Nil	Nil	Nil	Nil
Treated Milk	Nil	-	-	-	-	-	-	-	-	-	-	-
Dahi††	Nil	-	-	-	Nil	-	-	-	-	-	-	-
Chakka	Nil	-	-	-	Nil	-	-	-	Nil	-	-	-
Shrikhand	Nil	Nil	Nil	Nil	Nil	22	32	62	Nil	Nil	Nil	Nil

Note: Escherichia coli, Salmonella and Listeria monocytogens were not detected during storage.
†*Dahi* obtained by culturing milk inoculated with eXact Dahi 2+YoFlex Express 1.0 and incubated at 45°C/5h.
††*Dahi* obtained by culturing milk inoculated with JOINTEC VB530 and incubated at 45°C/5h.

JOINTEC VB530 in *shrikhand* spread with lower TS content (36.95%) than eXactDahi 2 + YoFlex Express 1.0 in *shrikhand* spread with higher TS content (43.84%). Karthikeyan *et al*. (2001) [52] reported that both the level of TS and storage period had a combined effect on the growth and activity of starter cultures at a particular storage temperature. However, Dhotre and Bhadania (2016) [53] could not establish any effect of TS content on acid development in *shrikhand* and recorded an elevation of titratable acidity from 1.05 to 1.21 % lactic acid after 15 days of storage at 8±2 °C.

Microbiological Quality of *Shrikhand* Spread

Coliform, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Listeria monocytogens* could not be detected in any of the fresh samples of *dahi*, *chakka* and *shrikhand* spread or during storage irrespective of the starter cultures employed (Table **3**). Yeast and moulds were not found in fresh samples of *dahi*, *chakka* and *shrikhand* spread but appeared in *shrikhand* spread after 3 days of storage regardless of starter combinations used. Results of the present investigation were in accordance with previous research [52,54]. Sarkar and Misra (1996a) [55] also could not detect *E. coli*, yeast and mould in fresh samples of *dahi*, however the organisms were found in *chakka* and *shrikhand*. Researchers suggested prevention of contamination of *chakka* and *shrikhand* spread from muslin cloth, environmental air and sugar. Better microbiological quality of *chakka* and *shrikhand* spread noted in the present investigation might be due pre-sterilization of polythene filter bags and admixturing of boiled sugar syrup instead of granular sugar without any bactericidal treatment. Greater increment in yeast and mould counts were observed in *shrikhand* spread made utilizing JOINTEC VB530 (22 to 62 cfu/ml) than those recorded for eXactDahi 2 + YoFlex Express 1.0 (14 to 32 cfu/ml) during 7 days of storage at 8±1°C, however, all samples of *shrikhand* spread were within the microbiological specifications of FSSAI (2015) [18]. Results indicated greater antibacterial activity of *shrikhand* spread made with eXactDahi 2 + YoFlex Express 1.0 than with JOINTEC VB530 towards yeasts and moulds.

Greater whey expulsion, lower acidity and higher total solids (43.84%) in *shrikhand* spread made with eXactDahi 2 + YoFlex Express 1.0 than with JOINTEC VB530 indicated that the antibacterial activity was due to elaboration of antimicrobial compounds by the starter combination eXactDahi 2 + YoFlex Express 1.0 and not due to lactic acid. Earlier studies indicated antibacterial activity of *shrikhand* was due to lactic acid (Sarkar and Misra, 1996 b) [56] due to lower retention of lactic acid bacteria in *chakka* and greater expulsion in *chakka* whey (Sarkar and Misra, 1996 a) [55].

CONCLUSION

Traditional method of *dahi* production with undefined starter cultures and uncontrolled fermentation is known to be associated with non-specific long curdling time and non uniform product quality. Conjugated use of yoghurt cultures with *dahi* cultures found to enhance dietetic value besides reducing the production time in *dahi*. Seeding of milk

with a starter combination of eXactDahi 2 + YoFlex Express 1.0 and an incubation temperature-time combination of 45°C/5h was found most suitable for producing *dahi* with hard smooth body, highest volatile acidity and low syneresis. Utilization of *dahi* obtained employing the above starter combination for *shrikhand* spread was found to furnish a shelf-life of 7 days at 8±1°C. Higher shelf-life of *shrikhand* spread with such starters, temperature-time combination and storage ambience is suggested for better quality *shrikhand* spread coupled with an extended market reach.

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