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 expolysaccharides (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.](image-url)
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 8°C 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 monocytogenes 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 701 was 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 | Titratable Acidity (% Lactic acid) | Syneresis (%) | Volatile Acidity (ml of 0.1N NaOH/50g curd) | Rheological Properties |
|----------------------|-----------------------|-----------------------------------|---------------|---------------------------------------------|------------------------|
| Yoghurt NCDC-144     | 42°C/5h               | 0.46                              | -             | -                                           | Partial Curdling       |
| Dahi NCDC-167        | 30°C/5h               | 0.70                              | 0.72          | 0.80                                         | Soft body              |
| Dahi NCDC-159        | 30°C/5h               | 0.70                              | 0.77          | 0.78                                         | Soft body              |
| JOINTEC VB530        | 37°C/5h               | 0.74                              | 0.98          | 1.04                                         | Ropiness noticed       |
|                      | 45°C/5h               | 0.75                              | 0.96          | 0.92                                         | Slightly soft body     |
| STA IDC 701          | 37°C/5h               | 0.55                              | 0.83          | 0.80                                         | Bitter taste           |
|                      | 42°C/4h               | 0.68                              | 0.88          | 0.85                                         | Bitter taste           |
|                      | 42°C/5h               | 0.72                              | 12.29         | 0.83                                         | Slightly Salty         |
| LYO DAHI             | 45°C/5h               | 0.59                              | 0.83          | -                                           | Higher Syneresis       |
| YO-MIX 336           | 45°C/5h               | 0.71                              | 0.87          | -                                           | Higher Syneresis       |
| YoFlex Express 1.0   | 42°C/4h               | 0.62                              | 0.74          | 0.74                                         | No whey separation     |
| eXact Dahi 2         | 37°C/5h               | 0.60                              | 0.70          | 0.70                                         | Hard body, Grainy      |
| STI-13               | 42°C/4h               | 0.67                              | 0.72          | 0.74                                         | Grainy body            |
| eXact Dahi2 +        | 42°C/4h               | 0.62                              |              |                                             | Very Soft body         |
| Yoghurt NCDC-144 +STI-13 | 42°C/4h            |                                      |               |                                             |                       |
| JOINTEC VB530 +STA IDC 701 | 42°C/4h      |                                      |               |                                             |                       |
| YoFlex Express 1.0+ STI-13 | 42°C/4h     |                                      |               |                                             |                       |
| STI-13+ CHN-11       | 37°C/5h               | 0.59                              | 0.71          | 0.72                                         | Slightly weak body, No grains |
| eXact Dahi2 +        | 44°C/5h               | 0.83                              | 1.02          | 1.03                                         | Soft body, Ropiness noticed |
| YoFlex Express 1.0+ STI-13 | 42°C/5.25h         |                                      |               |                                             |                       |
|                     | 42°C/5.5h             | 0.79                              | 1.03          | -                                           | Grainy texture         |
| STI-13+ CHN-11       | 37°C/5h               | 0.82                              | 0.94          | 0.91                                         | Grainy texture         |
| eXact Dahi2 +        | 44°C/5h               | 0.83                              | 1.04          | 1.04                                         | Soft body, Ropiness noticed |
| YoFlex Express 1.0+ STI-13 | 42°C/5.50h          |                                      |               |                                             |                       |
|                     | 43°C/4h               | 0.63                              | 0.68          | 0.72                                         | Slightly Soft body, Ropiness noticed |
| eXact Dahi2 +        | 42°C/4h               | 0.84                              | 0.91          | 0.91                                         | Slightly Salty         |
| YoFlex Express 1.0   | 42°C/5h               | 0.82                              | 0.92          | 0.92                                         | Slightly Soft body     |
|                     | 45°C/5h               | 0.88                              | 0.93          | 0.93                                         | Hard smooth body       |

storage. However, slightly higher values for volatile acidity (0.62 vs. 0.73 ml of 0.1N NaOH/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

| 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 2.9x10^9cfu/ml), with an increase in acidity (0.58 to 0.66 % lactic acid) during 8 days storage at 16-24°C [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
JOINTEC VB530 in shrikhand spread with lower TS content (36.95%) than eXactDahi 2 + YoFlex Express 1.0 in 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 or during storage irrespective of the starter cultures used. Results of the present investigation were in accordance with previous research [52,54]. Sarkar and Misra (1996 a) [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.

**Microbiological Quality of Shrikhand Spread**

Coliform, Staphylococcus aureus, Escherichia coli, Salmonella and Listeria monocytogenes 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 (1996 a) [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.

**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

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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 monocytogenes were not detected during storage.

†Dahi obtained by culturing milk inoculated with eXactDahi 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.
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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Received on 06-04-2018 Accepted on 23-04-2018 Published on 30-04-2018

DOI: https://doi.org/10.6000/1929-5634.2018.07.01.3