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Challenges Associated with the Development of Lactic Acid Bacteria and Probiotic Containing Fermented Shrikhand

G. SWAPNA, G. P. BRAHMAPRAKASH AND SUVARNA V. CHAVANNAVAR

Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK Bangalore 560065, India
E-mail : sapnamicro@rediffmail.com

Abstract

The investigation was conducted to study the sensory quality of shrikhand by the addition of probiotics and lactic acid bacteria as a starter culture, instead of using curds as a starter culture. The most commonly used probiotics are *Lactobacillus acidophilus, Lactobacillus sporogens* and *Lactobacillus rhamnosus* and in combinations like *Lactobacillus acidophilus* + *Lactobacillus sporogens*. The different lactic acid bacterial isolates were obtained from cattle milk, buffalo milk and dairy milk also used as starter culture singly or in combination with probiotics. The shrikhand samples were tested for organoleptic characteristics of the product. Results revealed that in the shrikhand prepared using probiotics as starter culture, the highest score was recorded with combinations of probiotics i.e. *Lactobacillus acidophilus* + *Lactobacillus sporogens*, and the lowest score was recorded in *Lactobacillus rhamnosus* in terms of color, appearance, aroma, texture, taste and overall acceptability of the product. The same results were obtained in addition of lactic acid bacterial isolates along with probiotics.
Key words : Shrikhand, Milk, Starter cultures, Lactic acid bacteria (LAB), Probiotics.

Modern consumers are increasingly interested in their personal health. Dietetic significance of Indian fermented milk products has been established due to its various nutritional and therapeutic properties. Biotechnological innovations suggest inclusion of certain lactic acid bacteria for further enhancement in the dietetic properties of traditional dairy products. Fermented foods are of great significance since they provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aroma and textures, which enrich the human diet. Shrikhand is an indigenous, concentrated, sweetened, and lactic fermented milk product prepared with species of *Lactobacillus* and *Streptococcus*, popularly and widely consumed as sweet dish in Maharashtra, Gujarat and parts of Karnataka. Shrikhand is considered the traditional Indian fermented milk product. Microbial cultures have been used for thousands of years in food and alcoholic fermentations, and in the past century have undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. This has led to the coining of the term probiotics. Today probiotics are available in a variety of food products and supplements, and have got wide applications in the control of cholestrol, cancers, allergies. Probiotics are live microbial food supplements, which benefit the health of consumers by maintaining, or improving their intestinal microbial balance. Therefore, the present study was undertaken with the objective to study the effect of probiotics as starter cultures on the quality of shrikhand, in comparison with that of lactic acid bacterial isolates.

Methods

The three different types of milk i.e. cattle milk, buffalo milk and the commercially available dairy milk were used in the preparation of shrikhand. The lactic acid bacteria (LAB) was isolated from shrikhand prepared from different types of milk. Thus, obtained LAB isolates used as starter culture. Similarly, three different commercially available probiotics i.e. *Lactobacillus acidophilus, Lactobacillus sporogenes* and *Lactobacillus rhamnosus* are used singly or in combination. The lactic acid bacterial isolates obtained from three different types of milk also used singly or in combination with probiotics during the preparation of shrikhand.

Table 1. Sensory evaluation of shrikhand. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely.

Milk samples	Appearance	Aroma	Texture	Taste	Overall acceptability
Cattle milk	4.8	4.8	4.8	4.7	4.6
Buffalo milk	4.7	4.6	4.6	4.5	4.4
Dairy milk	4.7	4.4	4.6	4.6	4.3

Isolation of Lactic Acid Bacteria from Shrikhand

Lactic acid bacteria (LAB) were isolated from shrikhand prepared using cattle milk, buffalo milk and commercially available dairy milk by standard plate count technique using Mann, Rogosa and Sharpe's (MRS) agar medium (1). Lactic acid bacterial colonies thus obtained were further purified and were maintained in MRS broth.

Preparation of Starter Culture

Fresh milk samples were heated and cooled to 30—32 C and then inoculated with commercially available probiotics, lactic acid bacterial isolates and curds as standard for 10—12 h till the sufficient acidity was developed, it was then used as starter culture for further fermentation process.

Table 2. Organoleptic evaluation of shrikhand prepared using lactic acid bacterial isolates as starter culture. LAB : Lactic acid bacteria. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely. B_1 : Lactic acid bacteria isolated from buffalo milk, D_1, D_2 & D_4 : Lactic acid bacteria isolated from dairy milk, C_1 C_3 & C_4 : Lactic acid bacteria isolated from cattle milk.

Treatments	Appearance	Aroma	Texture	Taste	Overall acceptability
C_1	3.8	3.8	3.9	3.7	3.8
C_3	3.8	3.8	3.7	3.8	3.8
C_4	3.8	3.9	3.3	3.8	3.9
B_1	3.6	3.4	3.6	3.6	3.2
D_4	3.8	3.5	3.3	3.5	3.5
D_2	3.7	3.5	3.2	3.5	3.7
D_1	3.7	3.7	3.6	3.6	3.6

Table 3. Organoleptic evaluation of probiotic (*Lactobacillus sporogenes*) added shrikhand. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely.

Milk samples	Appearance	Aroma	Texture	Taste	Overall acceptability
Cattle milk	3.9	3.8	3.7	3.8	3.8
Dairy milk	3.6	3.6	3.6	3.7	3.6
Buffalo milk	3.5	3.5	3.5	3.6	3.6

Preparation of Shrikhand from Cattle Buffalo and Dairy Milk

Fresh milk (cattle, buffalo and dairy) samples were used for shrikhand preparation. milk was heated to 95 C for 15 minutes, cooled to 37—40 C and inoculated with curd as starter culture. It was then incubated till coagulation or setting of curd. Whey was separated from curds by tieing the curds in a muslin cloth and hanging for eight hrs. The concentrated mass obtained after whey separation was used as the base material for the preparation of shrikhand. Chakka, thus obtained was mixed with powdered sugar and cardamom at 40 and 1.6% of chakka weight respectively. Similarly, shrikhand were prepared by using probiotics and lactic acid bacterial isolates as a starter culture singly or in combination.

Effect of Probiotics as Starter Culture on the Quality of Shrikhand Using Cattle Milk, Dairy Milk and Buffalo Milk

The most commonly used probiotics are *Lactobacillus sporogenes, Lactobacillus acidophilus* and *Lactobacillus rhamnosus* were obtained from commercially available probiotic sachets. Shrikhand was

Table 4. Organoleptic evaluation of probiotic (*Lactobacillus acidophilus*) added shrikhand. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely.

Milk samples	Appearance	Aroma	Texture	Taste	Overall acceptability
Cattle milk	4.2	4.3	4.0	4.2	4.2
Dairy milk	3.9	4.0	3.7	3.7	3.7
Buffalo milk	3.7	3.6	3.8	3.5	3.8

Table 5. Organoleptic evaluation of probiotic (*Lactobacillus rhamnosus*) added shrikhand. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely.

Milk samples	Appearance	Aroma	Texture	Taste	Overall acceptability
Cattle milk	2.6	2.7	2.4	2.4	2.6
Dairy milk	2.5	2.1	2.3	2.0	1.9
Buffalo milk	2.0	1.7	1.6	2.0	1.6

prepared by adding probiotics singly or in combinations such as *Lactobacillus sporogenes, L. acidophilus* and *L. rhamnosus* and *L. sporogenes* + *L. acidophilus* as starter cultures instead of using curd. Combinations of *L. sporogenes* + *L. rhamnosus, L. acidophilus* + *L. rhamnosus* and *L. sporogenes* + *L. rhamnosus* + *L. acidophilus* added shrikhand were not prepared as *L. rhamnosus* is an odd culture which produced bad smell.

Screening of Cattle, Buffalo and Dairy Milk and the Effect of Lactic Acid Bacterial Isolates and the Probiotics as a Starter Culture on the Quality of Shrikhand

Shrikhand was prepared using cattle milk, buffalo milk and dairy milk by adding curds as inoculum. Similarly, shrikhand was made by adding lactic acid bacterial isolates and probiotics as starter culture. Thus, prepared shrikhand samples were evaluated with respect to organoleptic characteristics like appearance, aroma, taste, texture and overall acceptability by a panel of ten members.

Organoleptic Evaluation

All shrikhand samples were evaluated by a selected panel of 10 members, which was based mainly on the appearance, color, aroma, taste and overall acceptability of the product.

Table 6. Organoleptic evaluation of probiotic (*Lactobacillus sporogenes* + *Lactobacillus acidophilus*) added shrikhand. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely.

Milk samples	Appearance	Aroma	Texture	Taste	Overall acceptability
Cattle milk	4.6	4.5	4.3	4.5	4.4
Dairy milk	4.2	4.0	4.2	3.8	3.8
Buffalo milk	3.7	3.6	3.6	3.6	3.6

Table 7. Organoleptic evaluation of (*Lactobacillus sporogenes*) and lactic acid bacterial isolates added shrikhand. LAB : Lactic acid bacteria. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely. B_1 : Lactic acid bacteria isolated from buffalo milk. D_1, D_2 & D_4 : Lactic acid bacteria isolated from dairy milk. C_1, C_3 & C_4 : Lactic acid bacteria isolated from cattle milk.

Treatments	Appearance	Aroma	Texture	Taste	Overall acceptability
C_1	4.1	3.9	3.7	3.9	3.7
C_3	3.9	3.7	3.7	3.8	4.0
C_4	3.7	3.8	4.0	3.9	3.7
B_1	3.7	3.7	3.7	3.8	3.3
D_4	3.7	3.8	3.7	3.7	3.6
D_2	3.6	3.8	3.6	3.7	3.6
D_1	3.7	3.6	3.6	3.5	3.8

Sample Presentation for Organoleptic Evaluation

Prior to tasting, each sample was coded and placed in a random manner, different shrikhand samples were placed along with water (to rinse the mouth) in the laboratory, and panelists were instructed

Table 8. Sensory evaluation of Shrikhand prepared using probiotic (*Lactobacillus acidophilus*) and lactic acid bacterial isolates as starter culture. LAB : Lactic acid bacteria. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely. B_1 : Lactic acid bacteria isolated from buffalo milk. D_1, D_2 & D_4: Lactic acid bacteria isolated from dairy milk. C_1, C_3 & C_4 : Lactic acid bacteria isolated from cattle milk.

Treatments	Appearance	Aroma	Texture	Taste	Overall acceptability
C_1	4.4	4.7	4.4	4.4	4.4
C_3	4.5	4.5	4.5	4.5	4.5
C_4	4.5	4.6	4.5	4.5	4.6
B_1	4.1	4.2	4.4	4.4	4.3
D_4	3.8	4.3	4.4	4.3	4.1
D_2	3.9	4.4	4.3	4.3	4.3
D_1	4.1	4.4	4.3	4.4	4.0

Table 9. Sensory evaluation of (*Lactobacillus rhamnosus*) and lactic acid bacterial isolates added to shrikhand. LAB : Lactic acid bacteria. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely. B_1 : Lactic acid bacteria isolated from buffalo milk. D_1, D_2 & D_4 : Lactic acid bacteria isolated from dairy milk. C_1, C_3 & C_4 : Lactic acid bacteria isolated from cattle milk.

Treatments	Appearance	Aroma	Texture	Taste	Overall acceptability
C_1	3.7	2.4	2.4	2.4	2.4
C_3	3.5	2.7	2.2	2.8	2.5
C_4	3.6	2.5	2.2	2.4	2.3
B_1	3.8	2.4	2.3	2.3	2.6
D_1	3.8	2.4	2.6	2.4	2.4
D_2	3.1	2.5	2.5	2.5	2.2
D_1	3.6	2.3	2.5	2.2	2.3

Table 10. Sensory evaluation of Shrikhand prepared using probiotic (*Lactobacillus sporogenes + Lactobacillus acidophilus*) and lactic acid bacteria as starter culture. LAB : Lactic acid bacteria. Each value is an average of 10 replications (10 persons). Scale : 5-Liked extremely; 4-Liked; 3-Neither liked nor disliked; 2-Disliked; 1-Disliked extremely. B_1 : Lactic acid bacteria isolated from buffalo milk, D_1 D_2 & D_4 : Lactic acid bacteria isolated from dairy milk. C_1, C_3 & C_4 : Lactic acid bacteria isolated from cattle milk.

Treatments	Appearance	Aroma	Texture	Taste	Overall acceptability
C_1	4.8	4.5	4.6	4.6	4.5
C_3	4.5	4.4	4.5	4.7	4.3
C_4	4.4	4.6	4.6	4.1	4.4
B_1	4.6	4.4	4.4	4.4	4.3
D_4	4.4	4.5	4.8	4.3	4.5
D_2	4.4	4.6	4.5	4.4	4.4
D_1	4.7	4.3	4.6	4.5	4.5

to evaluate each sample by blind tasting as per the standard score card for organoleptic evaluation.

Results and Discussion

Sensory Evaluation of Developed Products

The mean ranks of shrikhand prepared using cattle milk, buffalo milk and dairy milk with respect to appearance, aroma, texture, taste and overall acceptability are given in Table 1. Results revealed that highest score was recorded in shrikhand prepared using cattle milk (4.60 out of 5.00) and the lowest score was recorded in shrikhand prepared using dairy milk (4.30 out of 5.00) in terms of color, appearance, aroma, texture, taste and overall acceptability of the product. The shrikhand prepared using cattle milk recorded highest score, may be due to the graininess and curdiness and also due to the addition of sugar acting as a sweetening agent. Shrikhand prepared using dairy milk and buffalo milk scored the lowest, due to the its non-palatability, coarse, grainy, lumpy, curdy and dull look. The results obtained from the present investigation are in concurrence with the findings of Salunke et al. (2). The overall acceptability of shrikhand prepared using cattle milk recorded highest scores, this might be due to its semi-solid consistency and a characteristic firmness and pliability contributing to its suitability for consumption and the lowest scores were found in buffalo milk due to its coarse texture and graininess. Similar results were reported by Rameshwar (3).

Organoleptic Evaluation of Shrikhand Prepared Using Lactic Acid Bacterial Isolates as Starter Culture

Shrikhand prepared using cattle milk, buffalo milk and dairy milk with addition of lactic acid bacterial (LAB) isolates i.e. B_1 (lactic acid bacteria isolated from buffalo milk), D_1, D_2 and D_4 (lactic acid bacteria isolated from dairy milk) and C_1, C_3 and C_4 (lactic acid bacteria isolated from cattle milk) as a starter are given in Table 2. The shrikhand samples prepared using lactic acid bacterial isolates were tested for organoleptic characteristics of the product. Results revealed that, the highest score was recorded with LAB isolates i.e. C_1, C_3 and C_4 obtained from cattle milk (3.80, 3.80 and 3.90 out of 5.00), followed by lactic acid bacteria isolates obtained by buffalo milk i.e. B_1 (3.20 out of 5.00) in terms of color, appearance, aroma, texture, taste and overall acceptability of the product. Similar observation was recorded by Rameshwar (3), who also reported shrikhand has a typical semi-solid consistency showing a characteristic firmness and pliability contributing to its suitability for consumption with puri and bread. Similar results were obtained by Salunke et al. (2). Jain et al. (4) and Patel and Chakraborty (5) who reported that various levels of

fat, moisture and sugar had the least effect on color but had a profound effect on appearance, flavor, body and textural properties of shrikhand. Similar findings were also reported by Rao et al. (6) that the lactic acid bacteria employed in the manufacture of fermented product altered the sensory characteristics and thereby improved the nutritive and commercial value of raw milk.

Organoleptic Evaluation of Shrikhand Obtained Using Addition of Probiotics as Starter Culture i.e. Singly or in Combination

The most commonly used probiotics are *Lactobacillus acidophilus, Lactobacillus sporogenes* and *Lactobacillus rhamnosus* and in combinations like *Lactobacillus acidophilus + Lactobacilus sporogenes.*

The shrikhand samples were tested for organoleptic characteristics of the product are given in Tables 3, 4, 5 and 6. Results revealed that in the shrikhand prepared using probiotics as starter culture, the highest score was recorded with combinations of probiotics i.e. *Lactobacillus acidophilus + Lactobacillus sporogenes in* cattle milk followed by dairy milk and buffalo milk (4.40, 3.80 and 3.60 out of 5.00) and in terms of color, appearance, aroma, texture, taste and overall acceptability of the product and the lowest score was recorded in shrikhand prepared using *Lactobacillus rhamnosus* in cattle milk followed by dairy milk and buffalo milk (2.60, 1.90 and 1.60 out of 5.00) in terms of color, appearance, aroma, texture, taste and overall acceptability of the product. These results are in conformation with the findings of Bajad et al. (7); Nalawade (8); Desai and Gupta (9) and Sarkar et al. (10).

Organoleptic Evaluation of Probiotics and Lactic Acid Bacterial Isolate Added Shrikhand

Shrikhand prepared by addition of probiotics singly or in combination i.e. *Lactobacillus acidophilus, Lactobacillus sporogenes* and *Lactobacillus rhamnosus* and in combinations like *Lactobacillus acidophilus + Lactobacillus sporogenes* along with different lactic acid bacterial isolates obtained from three different types of milk i.e. B_1 (lactic acid bacteria isolated from buffalo milk), D_1 D_2 and D_4 (lactic acid bacteria isolated from dairy milk) and C_1, C_3 and C_4 (lactic acid bacteria isolated from cattle milk) are given in Tables 6. 7, 8 and 9. Result revealed that the highest score was recorded with combinations of probiotics i.e. *Lactobacillus acidophilus + Lactobacillus sporogenes* along with lactic acid bacterial isolates in cattle milk i.e. C_1, C_3 and C_4 (4.50, 4.30 and 4.40 out of 5.00), the lowest score was recorded in shrikhand prepared using *Lactobacillus rhamnosus* + lactic acid bacterial isolates in case of dairy milk isolates i.e. D_1, D_2 and D_4 (2.20, 2.30 and 2.40 out of 5.00) in terms of color, appearance, aroma, texture, taste and overall acceptability of the product. Similar results were also obtained by Salunke et al. (2), Jain et al. (4) and Patel and Chakraborty (5) who reported that various levels of fat, moisture and sugar had the least effect on color but had a profound effect on appearance, flavor, body and textural properties of shrikhand.

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