

## Probiotics Status on Keeping Quality of Lactic Acid Bacteria Added Shrikhand and *In-Vitro* Testing of Antimicrobial Activity

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### Abstract

The investigation was conducted to study the effect of probiotics compared to that of lactic acid bacterial isolates. Shrikhand prepared using probiotics singly or in combination and also along with lactic acid bacterial isolates as a starter culture instead of using curd as starter culture. Shrikhand prepared using probiotics and lactic acid bacterial isolates were analyzed for pH, residual sugars, total sugars and for titrable acidity. The antimicrobial activity of shrikhand was tested against *Escherichia coli*, *Salmonella* sp. and *Staphylococcus aureus*. Results showed that the shrikhand prepared using probiotics in combination and with lactic acid bacterial isolates showed highest pH, residual sugars, total sugars and for titrable acidity compared to single probiotics with lactic acid bacterial isolates. *Salmonella* sp. *Escherichia coli* and *Staphylococcus aureus* was inhibited by  $L \times C_1 \times D_4$ ,  $S \times C_1$  and  $L \times C_4$ ,  $S \times L \times D_2$  and  $L \times C_4$ ,  $S \times L \times B_1$  respectively.

**Key words :** Lactic acid bacteria, Starter cultures, Fermented milk product, Probiotics, Pysico-chemical composition.

Shrikhand is the indigenous fermented milk product prepared by the fermentation of milk by using known strain of lactic acid bacteria. Shrikhand is extensively used as a sweet dish after meals. It is also used as a festive sweet in India. Sugar is added as additive to the Shrikhand to enhance taste and does not have any preservative effect. Other natural additives like dried fruits are added to the shrikhand to enhance flavor. Shrikhand is traditionally made at home in western India. The name shrikhand is derived from the Sanskrit work "Shikharini". Lactic acid bacteria refers to a large group of beneficial bacteria that have similar properties and all produce lactic acid as an end product of the fermentation process. They are widespread in nature and are also found in our digestive systems. Although they are best known for their role in the preparation of fermented dairy products. Lactic acid also gives fermented milks their slightly tart taste. Additional characteristic flavors and aromas are often the result of other products of lactic acid. Lactic acid bacteria are therefore excellent ambassadors for an often maligned microbial world. They are not only of major economic significance, but are also of value in maintaining and promoting human health. Probiotic fermented milks, is one major seg-

ment amongst fermented milks that has tremendous potential for growth and development. Milk is an excellent medium to carry or generate live and active cultured dairy products. The technology of application of probiotic organisms in fermented dairy products aims to combine the potential health benefits of the bacteria with their ability to grow in milk, resulting in a nutritionally healthy and desirable product for the consumers. Shrikhand consumed as taste enhancer are produced by lactic acid fermentation, where in lactic acid bacteria ferment the milk and yield value added product with enhanced nutrients. Fermentation process not only enhances the nutritional value, but, the product gets enriched with probiotic organisms mainly lactic acid bacteria. Lactic acid bacteria produce several antibacterial compounds which can be used to claim health benefits especially in controlling enteric pathogens.

### Methods

#### *Isolation of Lactic Acid Bacteria from Shrikhand*

Lactic acid bacteria (LAB) were isolated from shrikhand prepared using cattle milk buffalo milk and

**Table 1.** Titrable acidity (%) of shrikhand samples prepared using lactic acid bacterial isolates with probiotics. C : Control, P<sub>1</sub> : *Lactobacillus sporogenes*, P<sub>2</sub> : *L. acidophilus*, P<sub>3</sub> : *L. rhamnosus*, P<sub>1</sub> × P<sub>2</sub> : *L. sporogenes* + *L. acidophilus*, B<sub>1</sub> : Lactic acid bacteria isolated from buffalo milk, D<sub>1</sub>, D<sub>2</sub> & D<sub>4</sub> : Lactic acid bacteria isolated from dairy milk, C<sub>1</sub>, C<sub>3</sub> & C<sub>4</sub> : Lactic acid bacteria isolated from cattle milk.

Treatments	Titrable acidity (%)					Mean
	Lactic acid bacterial isolates					
	C	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>1</sub> × P <sub>2</sub>	
C <sub>1</sub>	1.21	1.24	1.22	1.23	1.24	1.23
C <sub>3</sub>	1.20	1.23	1.21	1.24	1.25	1.23
C <sub>4</sub>	1.17	1.26	1.20	1.22	1.30	1.24
D <sub>2</sub>	1.18	1.22	1.26	1.23	1.29	1.26
D <sub>4</sub>	1.20	1.25	1.24	1.26	1.28	1.25
D <sub>1</sub>	1.18	1.26	1.26	1.25	1.30	1.26
B <sub>1</sub>	1.22	1.29	1.27	1.29	1.30	1.28
Mean	1.21	1.25	1.23	1.24	1.26	
Source	SE ±		CD 5%			
Probiotics (P)	0.02		0.03			
Treatments (T)	0.03		0.05			
Interaction (P×T)	4.00		1.68			

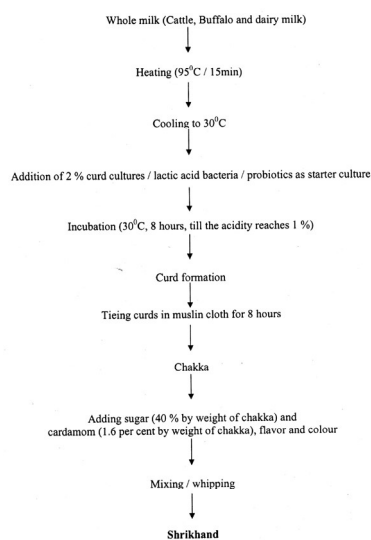
commercially available dairy milk by standard plate count technique using Mann, Rogosa and Sharpe's (MRS) agar medium (1). Lactic acid bacterial colonies thus obtained were further purified and were maintained in MRS broth. Fresh milk samples were heated and cooled to 30—32 C and then inoculated with curd as starter culture, then the shrikhand samples were subjected to serial dilution to obtain the lactic acid bacterial isolates. Thus, obtained lactic acid bacterial isolates were maintained in the MRS broth as pure culture. Then, the lactic acid bacteria isolates were further characterized for their biochemical changes.

#### Preparation of Starter Culture from Shrikhand

The lactic acid bacterial isolates obtained from three different types of milk viz., cattle milk (C<sub>1</sub>, C<sub>3</sub> and C<sub>4</sub>), buffalo milk (B<sub>1</sub>) and dairy milk (D<sub>1</sub>, D<sub>2</sub> and D<sub>4</sub>) were further purified and were maintained in MRS broth were uses as started culture for further preparation of shrikhand instead of using curd as starter culture, it was then used as starter culture for further fermentation process.

#### Probiotics

Different commercially available probiotics i.e.



**Figure 1.** Flow diagram illustrating the preparation of shrikhand.

*Lactobacillus acidophilus*, *Lactobacillus sporogenes* and *Lactobacillus rhamnosus* are used singly or in combination. Thus, 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.

#### Method of Preparation of Shrikhand

Fresh cattle milk, buffalo milk and dairy milk were pasteurized at 95 C for 15 minutes, cooled to 37—40 C. It is then inoculated with curd/lactic acid bacteria/probiotics as starter culture at 1% which is mixed well and incubated at 2830 C for 8—9 hours till coagulation or setting of curd (Fig. 1). When curd has set firmly (acidity 0.7—0.8% lactic), it is placed in a muslin cloth bag and removed the whey content after 8—9 hours. Now the curd get change into solid mass called Chakka. The concentrated mass i.e. Chakka, thus obtained after whey separation was used as the base material for the prepatation of shrikhand and blending with additives like sugar and cardamon at 40 and 1.6%, flavor and color. Thus, the conventional shrikhand making technology is a tedious and time consuming process.

**Table 2.** Total sugar (%) of shrikhand prepared using lactic acid bacterial isolates with probiotics. C : Control, P<sub>1</sub> : *Lactobacillus sporogenes*, P<sub>2</sub> : *L. acidophilus*, P<sub>3</sub> : *L. rhamnosus*, P<sub>1</sub> × P<sub>2</sub> : *L. sporogenes* + *L. acidophilus*, B<sub>1</sub> : Lactic acid bacteria isolated from buffalo milk, D<sub>1</sub>, D<sub>2</sub> & D<sub>4</sub> : Lactic acid bacteria isolated from dairy milk, C<sub>1</sub>, C<sub>3</sub> & C<sub>4</sub> : Lactic acid bacteria isolated from cattle milk.

Treatments	Total sugar (%)					Mean
	Lactic acid bacterial isolates					
	C	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>1</sub> × P <sub>2</sub>	
C <sub>1</sub>	50.95	54.07	54.70	53.57	54.86	53.85
C <sub>3</sub>	50.92	53.10	53.92	51.02	53.94	52.99
C <sub>4</sub>	50.89	53.16	52.93	53.75	54.18	53.67
D <sub>2</sub>	52.86	53.97	54.20	53.19	54.45	53.96
D <sub>4</sub>	52.05	53.12	54.75	54.80	54.94	54.10
D <sub>1</sub>	50.56	53.41	53.85	53.44	54.01	53.41
B <sub>1</sub>	52.79	54.69	54.94	54.96	55.07	54.62
Mean	51.57	53.64	54.18	53.53	54.49	
Source	SE ±		CD 5%			
Probiotics (P)	0.82		1.63			
Treatments (T)	1.25		2.49			
Interaction (P × T)	1.025		4.06			

#### Chemical Composition of Shrikhand

The shrikhand samples were analyzed for pH, titratable acidity, total sugars and reducing sugars.

#### pH

The shrikhand samples were tested for the pH. The pH of shrikhand samples was measured using digital pH meter of Anolog Model (Corin. Research USA).

#### Total Titrable Acidity

Samples (10g) were diluted with 30 ml of water and were titrated against 0.1 N NaOH using phenolphthalein as an indicator. The acidity was calculated by using the following formula and expressed in per cent according to Srivastava and Kumar (2).

$$\text{Total acid (\%)} = \frac{\text{Titre value} \times \text{N of alkali} \times \text{vol made up} \times \text{equivalent wt of acid} \times 100}{\text{Volume of the sample taken} \times 1000}$$

#### Reducing Sugar

It was estimated by following the method de-

**Table 3.** Residual sugars (%) of shrikhand prepared using lactic acid bacterial isolates with probiotics. C : Control, P<sub>1</sub> : *Lactobacillus sporogenes*, P<sub>2</sub> : *L. acidophilus*, P<sub>3</sub> : *L. rhamnosus*, P<sub>1</sub> × P<sub>2</sub> : *L. sporogenes* + *L. acidophilus*, B<sub>1</sub> : Lactic acid bacteria isolated from buffalo milk, D<sub>1</sub>, D<sub>2</sub> & D<sub>4</sub> : Lactic acid bacteria isolated from dairy milk, C<sub>1</sub>, C<sub>3</sub> & C<sub>4</sub> : Lactic acid bacteria isolated from cattle milk.

Treatments	Residual sugars (%)					Mean
	Lactic acid bacterial isolates					
	PC	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>1</sub> × P <sub>2</sub>	
C <sub>1</sub>	22.23	22.36	22.43	22.46	22.53	22.45
C <sub>3</sub>	22.16	22.30	22.48	22.44	22.60	22.43
C <sub>4</sub>	22.20	22.36	22.40	22.40	22.46	22.40
D <sub>2</sub>	22.23	22.33	22.36	22.40	22.44	22.42
D <sub>4</sub>	22.10	22.30	22.40	22.36	22.46	22.37
D <sub>1</sub>	22.24	22.36	22.36	22.38	22.54	22.44
B <sub>1</sub>	22.27	22.40	22.50	22.50	22.66	22.52
Mean	22.20	22.34	22.42	22.42	22.52	
Source	SE ±		CD 5%			
Probiotics (P)	0.13		0.26			
Treatments (T)	0.20		0.40			
Interaction (P × T)	0.02		0.10			

scribed by Shaffer-Somogyi micro method (3).

$$\text{Reducing sugars (\%)} = \frac{\text{Dextrose (mg)} \times \text{vol made up} \times 100}{5 \times \text{wt of sample taken} \times 1000}$$

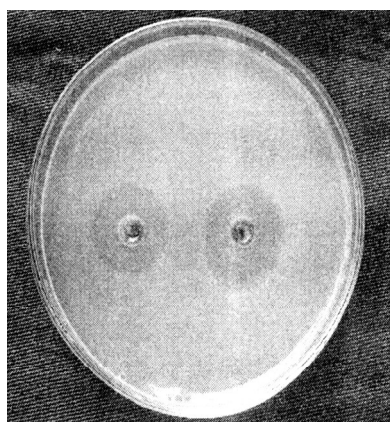
#### Total Sugars

A shrikhand sample of 0.1 ml was taken in a series of test tubes, added 1.0 ml of distilled water to each test tube. Pipetted out one ml of distilled water into another test tube to serve as blank. Added 0.5 ml of phenol reagent to each test tube and mixed well. Then five ml of sulphuric acid added to each test tube and allowed to stand for 10 min. The solution was then mixed well and placed in a water bath at 25—30 C for 10—20 min. The absorbance of the solution was recorded at 490 nm according to Dubios (4).

$$\text{Total sugar as invert sugar} = \frac{\text{Dextrose (mg)} \times \text{vol made up} \times 100}{\text{Titer value} \times \text{wt of sample taken} \times 1000}$$

$$\text{Sucrose (\%)} = (\% \text{ Total invert sugar} - \% \text{ reducing sugar}) \times 0.95$$

$$\text{Total sugars (\%)} = \% \text{ reducing sugar} + \% \text{ sucrose}$$



**Figure 2.** *Escherichia coli* inhibited by shrikhand using *Lactobacillus acidophilus* and isolate C<sub>4</sub>.

*In-Vitro Testing of Anti-Microbial Activity of the Products Against Salmonella sp., Escherichia coli and Staphylococcus aureus*

The antibacterial activity of products against faecal indicator *Escherichia coli*, food poisoning bacteria *Salmonella* sp. and *Staphylococcus aureus* was studied by agar gel diffusion assay technique according to Prabha (5). Melted and cooled nutrient agar was seeded with 24 hrs old cultures of faecal indicator and food poisoning bacterial isolates and was poured to petri plates, allowed to set for 3 h. Two wells were scooped out in the medium with the help of sterile cork borer and 0.5 ml of product was transferred to these wells, the plates were incubated for 48 h. The diameter of the well was measured. The area of the zone of inhibition was calculated by using the formula.

$$\text{Area of the zone of inhibition} = \pi (R + r) (R - r)$$

Where R = is the radius of the zone of the inhibition,  
r = is the radius of the well.

### Results and Discussion

#### *Chemical Composition of Shrikhand*

The pH, residual sugars, total sugars and titrable acidity of shrikhand prepared using cattle milk, buf-

**Table 4.** pH of shrikhand samples prepared using lactic acid bacterial isolates with probiotics. C : Control, P<sub>1</sub> : *Lactobacillus sporogenes*, P<sub>2</sub> : *L. acidophilus*, P<sub>3</sub> : *L. rhamnosus*, P<sub>1</sub> × P<sub>2</sub> : *L. sporogenes* + *L. acidophilus*, B<sub>1</sub> : Lactic acid bacteria isolated from buffalo milk, D<sub>1</sub>, D<sub>2</sub> & D<sub>4</sub> : Lactic acid bacteria isolated from dairy milk, C<sub>1</sub>, C<sub>3</sub> & C<sub>4</sub> : Lactic acid bacteria isolated from cattle milk.

Treatments	pH					Mean
	Lactic acid bacterial isolates					
	C	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>1</sub> × P <sub>2</sub>	
C <sub>1</sub>	3.43	3.54	3.58	3.47	3.59	3.54
C <sub>3</sub>	3.49	3.61	3.56	3.60	3.71	3.62
C <sub>4</sub>	3.50	3.63	3.53	3.61	3.66	3.60
D <sub>2</sub>	3.46	3.50	3.49	3.62	3.68	3.58
D <sub>4</sub>	3.55	3.59	3.65	3.64	3.72	3.66
D <sub>1</sub>	3.56	3.62	3.60	3.67	3.70	3.65
B <sub>1</sub>	3.60	3.67	3.69	3.70	3.73	3.69
Mean	3.51	3.52	3.58	3.54	3.68	

falo milk and dairy milk with different probiotics P<sub>1</sub> (*Lactobacillus sporogenes*), P<sub>2</sub> (*L. acidophilus*) and P<sub>3</sub> (*L. rhamnosus*) and in different combinations of P<sub>1</sub> × P<sub>2</sub> (*L. sporogenes* × *L. acidophilus*) along with different lactic acid bacterial isolates viz., cattle milk (C<sub>1</sub>, C<sub>3</sub> and C<sub>4</sub>), buffalo milk (B<sub>1</sub>) and dairy milk (D<sub>1</sub>, D<sub>2</sub> and D<sub>4</sub>) were estimated and are presented in (Tables 1, 2, 3 and 4). The treatments P<sub>1</sub> × P<sub>3</sub>, P<sub>2</sub> × P<sub>3</sub> and P<sub>1</sub> × P<sub>2</sub> × P<sub>3</sub> along with different lactic acid bacterial isolates are not presented in the result as they produced undesirable odor and not showed good quality with respect to appearance, taste, texture, color and overall acceptability, hence the treatments were discarded.

#### *Effect pH on Shrikhand*

The shrikhand prepared using lactic acid bacterial isolate C<sub>1</sub> had the highest pH in P<sub>1</sub> × P<sub>2</sub> (3.59) followed by P<sub>2</sub> (3.58), P<sub>1</sub> (3.54), P<sub>3</sub> (3.47) and the lowest pH was found in C (3.43), C<sub>3</sub> had the highest pH in P<sub>1</sub> × P<sub>2</sub> (3.71) followed by P<sub>1</sub> (3.61), P<sub>3</sub> (3.60), P<sub>2</sub> (3.56) and the lowest pH was found in C (3.49), C<sub>4</sub> had the highest pH in P<sub>1</sub> × P<sub>2</sub> (3.66) followed by P<sub>1</sub> (3.63), P<sub>3</sub> (3.61), P<sub>2</sub> (3.53) and the lowest pH was found in C (3.50), D<sub>2</sub> had the highest pH in P<sub>1</sub> × P<sub>2</sub> (3.68) followed by P<sub>3</sub> (3.62), P<sub>1</sub> (3.50), P<sub>2</sub> (3.49%) and the lowest pH was found in C (3.46), D<sub>4</sub> had highest pH was found in P<sub>1</sub> × P<sub>2</sub> (3.72) followed by P<sub>2</sub> (3.62), P<sub>3</sub> (3.65), P<sub>1</sub> (3.59) and the lowest pH was found in C (3.55), D<sub>1</sub> had the highest pH in P<sub>1</sub> × P<sub>2</sub> (3.70) followed by P<sub>3</sub> (3.67),

**Table 5.** *In vitro* testing of antimicrobial activity of the product against *Escherichia coli*, *Salmonella* sp. and *Staphylococcus aureus*. L : *Lactobacillus acidophilus*, S : *Sporolac (Lactobacillus sporogenes)*, C : Lactic acid bacterial isolates of cattle milk, D : Lactic acid bacterial isolates of dairy milk, B : Lactic acid bacterial isolates of buffalo milk, Values in the parantheses are square root transformed values.

Treatments	Area of zone of inhibition zone in sq mm		
	<i>E. coli</i>	<i>Salmonella</i> sp.	<i>Staphylococcus</i> <i>aureus</i>
1. L × C × D <sub>4</sub>	0.00 (0.71)	91.77 (9.61)	0.00 (0.71)
2. S × L × C <sub>3</sub>	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
3. S × L × D <sub>2</sub>	67.77 (8.26)	0.00 (0.71)	0.00 (0.71)
4. S × L × B <sub>1</sub>	0.00 (0.71)	0.00 (0.71)	71.50 (8.49)
5. S × L × C <sub>1</sub>	0.00 (0.71)	82.07 (9.08)	0.00 (0.71)
6. S × L × D <sub>4</sub>	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
7. L × C <sub>4</sub>	48.43 (6.99)	67.07 (8.22)	0.00 (0.71)
CD 5%	13.61	33.13	18.42

P<sub>1</sub> (3.62), P<sub>2</sub> (3.60) and the lowest pH was found in C (3.56) and B<sub>1</sub> had highest pH was found in P<sub>1</sub> × P<sub>2</sub> (3.73) followed by P<sub>2</sub> (3.58), P<sub>3</sub> (3.54), P<sub>1</sub> (3.52) and the lowest pH was found in C (3.60) This may be due to the conversion of lactose into lactic acid and other organic acids by the starter cultures that reduced the pH of shrikhand and the lactose content of shrikhand is dependent on the extent of lactose degradation, moisture content of shrikhand.

#### *Effect of Residual Sugars (%) on Shrikhand*

The shrikhand prepared using lactic acid bacterial isolate C<sub>1</sub> recorded highest residual sugars in P<sub>1</sub> × P<sub>2</sub> (22.53%) followed by P<sub>3</sub> (22.46%), P<sub>2</sub> (22.43%), P<sub>1</sub> (22.36%) and the lowest residual sugars was found in C (22.23%), C<sub>3</sub> had highest residual sugars was found in P<sub>1</sub> × P<sub>2</sub> (22.60%) followed by P<sub>2</sub> (22.48%), P<sub>3</sub> (22.44%), P<sub>1</sub> (22.30%) and the lowest residual sugars was found in C (22.16%), C<sub>4</sub> had the highest residual sugars was found in P<sub>1</sub> × P<sub>2</sub> (22.46%) followed by P<sub>2</sub> (22.40%), P<sub>3</sub> (22.40%), P<sub>1</sub> (22.36%) and the lowest residual sugars was found in C (22.20%), D<sub>2</sub> had highest residual sug-

ars was found in P<sub>1</sub> × P<sub>2</sub> (22.44%) followed by P<sub>3</sub> (22.40%), P<sub>2</sub> (22.36%) P<sub>1</sub> (22.33%) and the lowest residual sugars was found in C (22.23%), D<sub>4</sub> had highest residual sugars was found in P<sub>1</sub> × P<sub>2</sub> (22.46%) followed by P<sub>2</sub> (22.40%), P<sub>3</sub> (22.36%), P<sub>1</sub> (22.30%) and the lowest residual sugars was found in C (22.10%), D<sub>1</sub> recorded the highest residual sugars in P<sub>1</sub> × P<sub>2</sub> (22.54%) followed by P<sub>3</sub> (22.38%), P<sub>2</sub> (22.36%), P<sub>1</sub> (22.36%) and the lowest residual sugars (%) was found in C (22.24%) and B<sub>1</sub> had the highest residual sugars in P<sub>1</sub> × P<sub>2</sub> (22.66%) followed by P<sub>2</sub> (22.50%), P<sub>3</sub> (22.50%), P<sub>1</sub> (22.40%) and the lowest total sugars (%) was found in C (22.27%). This may be due to its influences in sugar utilization by lactic acid bacteria (6—10).

#### *Effect of Total Sugar (%) on Shrikhand*

The shrikhand prepared using lactic acid bacterial isolate C<sub>1</sub> had the highest total sugar in P<sub>1</sub> × P<sub>2</sub> (54.86%) followed by P<sub>2</sub> (54.70%), P<sub>1</sub> (54.07%), P<sub>3</sub> (53.57%) and the lowest total sugar was found in C (50.95%), C<sub>3</sub> had highest total sugar was found in P<sub>1</sub> × P<sub>2</sub> (55.07%) followed by P<sub>2</sub> (53.92%), P<sub>1</sub> (54.07%), P<sub>3</sub> (51.02%) and the lowest total sugar in C (50.92%), C<sub>4</sub> had highest total sugar was found on P<sub>1</sub> × P<sub>2</sub> (54.18%) followed by P<sub>3</sub> (53.75%), P<sub>1</sub> (53.16%), P<sub>2</sub> (52.93%) and the lowest total sugar was found in C (50.89%), D<sub>2</sub> had highest total sugar in P<sub>1</sub> × P<sub>2</sub> (54.45%) followed by P<sub>2</sub> (54.20%), P<sub>1</sub> (53.97%), P<sub>3</sub> (54.20%) and the lowest total sugar was found in C (52.86%), D<sub>4</sub> had highest total sugar in P<sub>1</sub> × P<sub>2</sub> (54.94%) followed by P<sub>3</sub> (54.80%), P<sub>2</sub> (54.75%), P<sub>1</sub> (53.12%) and the lowest total sugar was found in C (52.05%) D<sub>1</sub> had the highest total sugar was found in P<sub>1</sub> × P<sub>2</sub> (54.01%) followed by P<sub>2</sub> (53.85%), P<sub>3</sub> (53.44%), P<sub>1</sub> (53.41%) and the lowest total sugar was found in C (50.56%) and B<sub>1</sub> recorded the highest total sugar in P<sub>1</sub> × P<sub>2</sub> (55.07%) followed by P<sub>3</sub> (54.96%), P<sub>2</sub> (54.94%), P<sub>1</sub> (54.69%) and the lowest total sugar was found in C (52.79%). That may be due to the blending of sugar with chakka. Sugar slowed down the chemical changes due to fermentation (4, 8, 11—13).

#### *Effect of Titrable Acidity on Shrikhand*

The shrikhand prepared using lactic acid bacte-

rial isolate C<sub>1</sub> had highest titrable acidity in P<sub>1</sub> × P<sub>2</sub> (1.24%) followed by P<sub>1</sub> (1.24%), P<sub>3</sub> (1.22%) and the lowest titrable acidity was found in C (1.21%), C<sub>3</sub> had highest titrable acidity was found in P<sub>1</sub> × P<sub>2</sub> (1.25%) followed by P<sub>3</sub> (1.24%), P<sub>1</sub> (1.23%), P<sub>2</sub> (1.21%) and the lowest titrable acidity (%) was found in C (1.20%), C<sub>4</sub> had highest titrable acidity was found in P<sub>1</sub> × P<sub>2</sub> (1.30%) followed by P<sub>1</sub> (1.26%), P<sub>3</sub> (1.22%), P<sub>2</sub> (1.20%) and the lowest titrable acidity (%) was found in C (1.17%), D<sub>2</sub> had highest titrable acidity was found in P<sub>1</sub> × P<sub>2</sub> (1.29%) followed by P<sub>2</sub> (1.26%), P<sub>3</sub> (1.23%), P<sub>1</sub> (1.22%) and the lowest titrable acidity was found in C (1.18%), D<sub>4</sub> had highest titrable acidity in P<sub>1</sub> × P<sub>2</sub> (1.28%) followed by P<sub>3</sub> (1.26%), P<sub>1</sub> (1.25%), P<sub>2</sub> (1.24%) and the lowest titrable acidity was found in C (1.20%). In the shrikhand prepared using lactic acid bacterial isolate D<sub>1</sub>, the highest titrable acidity was found in P<sub>1</sub> × P<sub>2</sub> (1.30%) followed by P<sub>1</sub> (1.26%), P<sub>2</sub> (1.26%), P<sub>3</sub> (1.25%) and the lowest titrable acidity (%) was found in C (1.18%) and B<sub>1</sub> had the highest titrable acidity in P<sub>1</sub> × P<sub>2</sub> (1.30%) followed by P<sub>1</sub> (1.29%), P<sub>3</sub> (1.29%), P<sub>2</sub> (1.27%) and the lowest titrable acidity was found in C (1.22%). This may be due to gradual increase in titrable acidity with the storage period (10, 12, 14—17).

*In-Vitro Testing of Antimicrobial Activity  
of the Product Against Escherichia  
coil, Salmonella sp. and  
Staphylococcus aureus*

*In-vitro* testing of antimicrobial activity of shrikhand were presented in (Table 5). *Escherichia coli* was inhibited by two out of seven treatments. Highest inhibition was scored by S × L × D<sub>2</sub> (67.7 sq mm), followed by L × C<sub>4</sub> (48.43 sq mm). These two treatments differed significantly among themselves. *Salmonella* was inhibited by three out of seven treatments. The highest inhibition was scored by L × C × D<sub>4</sub> (91.77 sq mm), followed by L × C<sub>4</sub> (67.07 sq mm). These two treatments differed significantly between themselves. *Staphylococcus aureus* was inhibited by the treatment S × L × B<sub>1</sub> (71.50 sq mm) (5, 18—22).

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