Environment & Ecology 29 (3A) : 1326—1332, 2011
 ISSN 0970-0420

Development of Functional Food : Shrikhand

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Abstract

The investigation was conducted for characterization of lactic acid bacterial isolates from Shrikhand, a fermented dairy product. Shrikhand was prepared using cattle, buffalo and dairy milk by adding curd as starter culture for isolation of lactic acid bacterial isolates. These isolates were tested for Gram's reaction, colony morphology, and biochemical tests like catalase, fermentation of carbohydrates, gelatin hydrolysis, spore production, growth on neutral red chalk agar and dextran production. Result revealed that all the lactic acid bacterial isolates were Gram + ve, rods and cocci, catalase–ve, could not hydrolyze gelatin and they showed acid and gas production and isolates from dairy milk (D_2 and D_3) produced dextran.
Key words : Shrikhand, Probiotics, Total sugars, Residual sugars, Titrable acidity.

Fermented foods are of great significance since, they provide and preserve vast quantities of nutritious foods in a wide diversity of flavors, aromas and textures, which enrich the human diet. Over 3500 traditional, fermented foods exist worldwide. Fermented foods have been with us since humans arrived on earth and of these fermented milks have long been an important component of nutrition and diet. Fermented milk productes constitute a vital component of the human diet in many regions of the world. In the Indian sub-continent such products are also classified as "indigenous milk products" like dahi (curd), lassi, shrikhand which are prominent in people's diet. The viability and stability of probiotics has been both a marketing and technological challenge for industrial producers. The technological 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 is one such milk product which had its origin in Western India. The popularity of this product is mainly in the states of Gujarat, Maharashtra and Northern parts of Karnataka. It is obtained from curd by partial draining of whey, to which sugar, fruits and nuts are added. The product is popular especially in summer and served as a dessert in ceremonial functions. It has the nutritive goodness of fermented milk products. Like *dahi*, it is refreshing particularly during summer months. It is popular because of its characteristics flavor, taste, palatable nature and possible therapeutic value. Therefore, the present study was undertaken with the objective to study the effect of probiotics and lactic acid bacterial isolates as starter cultures on the quality of shrikhand by their physico-chemical characteristics.

Methods

Preparation of Shrikhand

Shrikhand was prepared by using three different types of milk i.e. cattle milk, buffalo milk and the commercially available dairy milk using curd as a starter culture and the different lactic acid bacterial isolates were obtained from shrikhand prepared using three different types of milk by using serial dilution method. Later, these lactic acid bacterial isolates obtained from shrikhand prepared from three different types of milk i.e. cattle milk (C_1, C_3 and C_4), buffalo milk (B_1) and dairy milk (D_1, D_2 and D_4) were maintained as pure culture and were used as starter culture for further preparation of shrikhand instead of using curd as starter culture. Similarly, the different commercially available probiotics i.e. *Lactobacillus acidophilus, Lactobacillus sporogenes* and *Lactobacillus rhamnosus* are used alone or in combination with each other as starter culture.

Fresh milk (cattle, buffalo and dairy) samples were

Table 1. Characterization of isolates from shrikhand. A : Acid production, C : LAB isolated from cattle milk, D : LAB isolated from dairy milk, B : LAB isolated from buffalo milk, G : Gas production and LAB : Lactic acid bacteria.

Isolates	Shape	Gram's reaction	Catalase activity	Glucose utilization A	Glucose utilization G	Dextran production	Gelatin hydrolysis	Spore production	Growth on neutral red chalk agar
C_1	Short rods	+	–	–	+	–	–	–	–
C_2	Short rods	+	–	–	–	–	–	–	–
C_3	Short rods	+	–	+	–	–	–	–	–
C_4	Long rods	+	–	–	+	–	–	–	–
B_1	Short rods	+	–	+	–	–	–	–	–
B_2	Long rods	+	–	+	–	–	–	–	–
B_3	Short rods	+	–	–	–	–	–	–	–
B_4	Short rods	+	–	–	–	–	–	–	–
D_1	Short rods	+	–	–	+	–	–	–	–
D_2	Cocci	+		+	–	+	–	–	+
D_3	Cocci	+	–	+	–	+	–	–	+
D_4	Long rods	+	–	+	–	–	–	–	–

used for shrikhand preparation. Milk was heated to 95 C for 15 minutes, cooled to 37—40 C and inoculated with curd/lactic acid bacteria/probiotics as starter culture (Fig. 1). It was then incubated till coagulation or setting of curd. Whey was separated from the curd by suspending for about eight hours in muslin cloth. The concentrated mass i.e. chakka, thus obtained after whey separation was used as the base material for the preparation 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. The products available in the market vary considerably in regard to their texture, organoleptic qualities and chemical composition.

Identification of Lactic Acid Bacterial Isolates

Lactic acid bacterial isolates obtained from shrikhand prepared out of three different types of milk were studied for their cell shape and growth characteristics on Mann, Rogosa and Sharpe's broth as suggested by Mann et al. (1). The gram staining was done using 24 hours old culture. The stained cells were observed under microscope according to Aneja (2).

Characterization of Lactic Acid Bacterial Isolates

Lactic acid bacterial isolates were characterized based on the growth in MRS broth using biochemical tests i.e. catalase production, fermentation of carbohydrates, spore production, dextran production, gelatin hydrolysis and growth on neutral red chalk agar medium.

Growth on Neutral Red Chalk Agar Medium

Neutral red chalk agar medium was poured to the petriplates and allowed for solidification. After solidification, isolates were streaked, then the plates were incubated at room temperature for 48 h and observations were recorded as suggested by Chalmers (3).

Fermentation of Carbohydrates

Isolates were inoculated in a test tube containing MRS broth with a specific carbohydrate i.e. glucose, the fermentation tube is a culture tube that contains Durham tube placed in an inverted position in the culture tube and a pH indicator for detection of gas production as suggested by Aneja (2).

Catalase Production

A loopful of 24 hrs old culture suspension was placed on a clean glass slide to which a drop of freshly prepared hydrogen peroxide (3%) was mixed and observed for the occurrence of gas bubbles as suggested by Aneja (2).

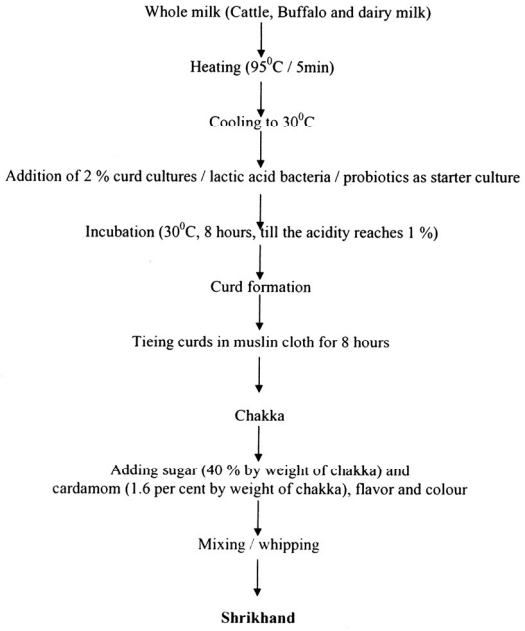


Figure 1. Flow diagram illustrating the preparation of shrikhand.

Dextran Production

Sucrose agar medium was poured to the petriplates and allowed for solidification. After solidification, isolates were streaked, then the plates were incubated at room temperature for 48 h and observations were recorded according to Garvie (4).

Gelatin Hydrolysis

Solidified plates of gelatin agar were streaked with culture and incubated at 37 C for 72 h. Further, the plates were flooded with 10 ml of $HgCl_2$ solution prepared by dissolving 15 g of mercuric chloride in 20 ml concentrated HCl which in turn was added to 100 ml distilled water and observed for the formation of clear zone around the growth according to Harrigan and Mc Cance (5).

Spore Production

Isolates were examined under microscope for spore production using malachite green. Smears were prepared using 15 days old broth cultures of isolates. Smears were flooded with malachite green, heated the slides to steaming on hot water bath and steamed for five minutes adding more stain to the smear from time to time, washed the slides slowly under running tap water, counter stained with safranin for 30 sec and washed the smears with distilled water then observed under microscope as suggested by Aneja (2).

Table 2. Titrable acidity (%) of shrikhand. C : Control, P_1 : *Lactobacillus sporogenes*, P_2 : *L. acidophilus*, P_3 : *L. rhamnosus*, $P_1 \times P_2$: *L. sporogenes* + *L. acidophilus.*

	Titrable acidity (%) Probiotics					
Treatments	C	P_1	P_2	P_3	$P_1 \times P_2$	Mean
Cattle milk	1.22	1.29	1.28	1.26	1.30	1.27
Buffalo milk	1.26	1.30	1.31	1.30	1.32	1.30
Dairy milk	1.21	1.25	1.24	1.24	1.28	1.25
Mean	1.22	1.28	1.27	1.26	1.30	
Source	SE ±				CD 5%	
Probiotics (P)	0.03				0.05	
Treatments (T)	0.02				0.04	
Interaction (P × T)	4.5				1.92	

Chemical Composition of Shrikhand

To assess the chemical composition of shrikhand, samples were analyzed for pH, titrable 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 (10 g) 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 (6).

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

Table 3. Total sugar (%) of shrikhand. C : Control, P_1 : *Lactobacillus sporogenes*, P_2 : *L. acidophilus*, P_3 : *L. rhamnosus*, $P_1 \times P_2$: *L. sporogenes* + *L. acidophilus.*

	Total sugar (%) Probiotics					
Treatments	C	P_1	P_2	P_3	$P_1 \times P_2$	Mean
Cattle milk	46.48	53.13	54.35	53.92	54.63	52.92
Buffalo milk	48.02	54.57	54.43	54.22	54.85	53.66
Dairy milk	46.48	51.26	53.52	50.90	53.72	51.81
Mean	46.99	52.98	54.10	53.01	54.40	
Source	SE ±			CD 5%		
Probiotics (P)	0.66			1.91		
Treatments (T)	0.94			1.35		
Interaction (P × T)	0.60			2.56		

Reducing Sugar

It was estimated by following the standard method described by Shaffer-Somogyi micro-method.

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

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 sulfuric 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 et al. (7).

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

Sucrose (%) = (% Total invert sugar – % reducing sugar) × 0.95

Total sugars (%) = % Reducing sugar + % Sucrose.

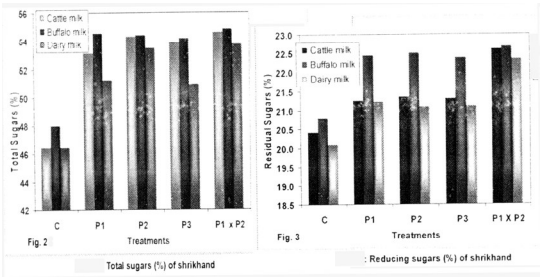
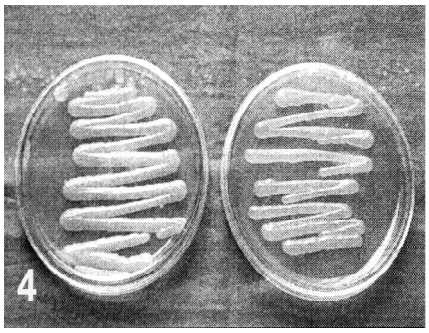


Figure 2. Total sugar (%) of shrikhand. **Figure 3.** Residual sugar (%) of shrikhand.

Results and Discussion

Characterization of Lactic Acid Bacterial Isolates from Shrikhand

All the test isolates formed characteristic white, submerged colonies on Mann, Rogosa and Sharpe's (MRS) agar medium. The lactic acid bacterial isolates were further examined for their shape and Gram reaction under microscope and their biochemical tests like catalase, fermentation of carbohydrates, growth on neutral red chalk agar medium, Gelatin hydrolysis, spore production and dextran production (Table 1, Figs. 2 to 5). Result revealed that, all the lactic acid bacterial isolates were Gram + ve, rods and cocci. The lactic acid bacterial isolates were catalase – ve, colonies appeared after 48 h on neutral red chalk agar, which were small, deep red, surrounded by a clear zone formed due to the dissolution of $CaCO_3$ in the medium (Fig. 5) could not hydrolyze gelatin and they showed that isolates C_3, B_1, B_2 D_2, D_3 and D_4 changed the color from red to yellow due to production of acids but C_1, C_4 and D_1 showed no change in color. Collection of gas in durham's tube was observed in the tubes inoculated with the isolates C_1, C_4 and D_1 acid and small colonies appeared after 24 h on sucrose agar which were creamish to yellowish in color. Isolates D_2 and D_3 could produce enormous deposition of slimy substances on the colonies, dextran was observed (Fig. 4). These findings support the reports of Kebede (8) reported that isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of Borde, an Ethiopian cereal beverage. Similar results were also reported by Rao et al. (9). Lactic acid bacteria can assimilate glu-



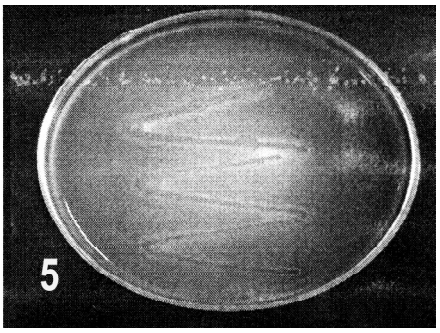


Figure 4. *Leuconostoc mesentroides* producing dextran on sucrose agar medium. **Figure 5.** Growth of *Lactococcus lactis* on neutral red agar medium.

cose as carbon source. All the isolates in this study showed assimilation of glucose in MRS broth. Glucose is a flavored source which is an indication of glycolysis and is a major carbon utilizing pathway. These results are similar to those of Samelis et al. (10) who also reported that gas (CO_2) production by glucose was determined in modified MRS broth containing inverted Durham tubes, with diammonium citrate replaced by ammonium sulfate.

Chemical Composition of Shrikhand

Titrable acidity, residual sugars, total sugars and pH of shrikhand prepared using cattle milk, buffalo milk and dairy milk with different probiotics P_1 (*Lactobacillus sporogenes*), P_2 (*L. acidophilus*) and P_3 (*L. rhamnosus*) and in different combinations of $P_1 \times P_2$ (*L. sporogenes* × *L. acidophilus*) were estimated and are presented in (Tables 2 to 5). The treatments $P_1 \times P_3$, $P_2 \times P_3$ and $P_1 \times P_2 \times P_3$ are not presented in the table as they produced undesirable odor and did not show good quality with respect to appearance, taste, texture, color and overall acceptability, hence the treatments were discarded.

Titrable Acidity

The titrable acidity in control (C), maximum was found in buffalo milk (1.26) followed by cattle milk (1.22) and dairy milk (1.21). In case of shrikhand pepared from probiotic *L. sporogenes* (P_1), maximum titrable acidity was found in buffalo milk (1.30) followed by cattle milk (1.29) and dairy milk (1.25). In probiotic *L. acidophilus* (P_2), maximum titrable acidity was found in buffalo milk (1.31) followed by cattle milk (1.28) and dairy milk (1.24). In probiotic *L. rhamnosus* (P_3), maximum titrable acidity was found in buffalo milk (1.30) followed by cattle milk (1.26) and dairy milk (1.24). In combination of probiotics *L. sporogenes* × *L. acidophilus* ($P_1 \times P_2$), maximum titrable acidity was found in buffalo milk (1.32) followed by cattle milk (1.30) and dairy milk (1.28).

In shrikhand prepared by using cattle milk, the highest titrable acidity was found in $P_1 \times P_2$ (1.30) followed by P_1 (1.29), P_2 (1.28), P_3 (1.26) and the lowest titrable acidity was found in C (1.22) and in the shrikhand prepared using buffalo milk, the highest titrable acidity was found in $P_1 \times P_2$ (1.32) followed by P_2 (1.31), P_3 (1.30), P_1 (1.30) and the lowest titrable acidity was found in C (1.24). Similarly, shrikhand prepared using dairy milk, the highest titrable acidity was found in $P_1 \times P_2$ (1.28) followed by P_1 (1.28), P_3 (1.24), P_2 (1.24) and the lowest titrable acidity was found in C (1.21). Titrable acidity is an important parameter to yield good quality of shrikhand. The results reported that highest titrable acidity was recorded in $P_1 \times P_2$ (*Lactobacillus sporogenes* + *L. acidophilus*) followed by P_1 (*L. sporogenes*). This may be due to gradual increase in titrable acidity with the storage period. These results are similar to those of Reddy et al. (11), Osman and Razig (12).

Total Sugar (%) of Shrikhand

The total sugar (%) in control (C), maximum was found in buffalo milk (48.02%) followed by cattle milk

Table 4. Residual sugars (%) of shrikhand. C : Control, P_1 : *Lactobacillus sporogenes*, P_2 : *L. acidophilus*, P_3 : *L. rhamnosus*, $P_1 \times P_2$: *L. sporogenes* + *L. acidophilus.*

Residual sugars (%)						
	Probiotics					
Treatments	C	P_1	P_2	P_3	$P_1 \times P_2$	Mean
Cattle milk	20.43	21.26	21.36	21.31	22.59	21.56
Buffalo milk	20.80	22.43	22.50	22.36	22.65	22.15
Dairy milk	20.10	21.23	21.10	21.11	22.33	21.51
Mean	20.44	21.64	21.65	21.59	22.52	
Source	SE ±			CD 5%		
Probiotics (P)	0.16			0.33		
Treatments (T)	0.23			0.46		
Interaction (P×T)	0.46			0.15		

Table 5. pH of shrikhand. C : Control, P_1 : *Lactobacillus sporogenes*, P_2 : *L. acidophilus*, P_3 : *L. rhamnosus*, $P_1 \times P_2$: *Lactobacillus sporogenes* + *Lactobacillus acidophilus.*

pH						
	Probiotics					
Treatments	C	P_1	P_2	P_3	$P_1 \times P_2$	Mean
Cattle milk	3.46	3.51	3.53	3.51	3.60	3.53
Buffalo milk	3.49	3.57	3.55	3.52	3.69	3.59
Dairy milk	3.48	3.52	3.49	3.50	3.58	3.54
Mean	3.47	3.53	3.52	3.51	3.62	

(46.48%) and dairy milk (46.48%). In probiotic *L. sporogenes* (P_1), maximum total sugar was found in buffalo milk (54.57%) followed by cattle milk (53.13%) and dairy milk (51.26%). In probiotic *L. acidophilus* (P_2), maximum total sugar was found in buffalo milk (54.43%) followed by cattle milk (54.35%) and dairy milk (53.52%). In probiotic *L. rhamnosus* (P_3), maximum total sugar was found in buffalo milk (54.22%) followed by cattle milk (53.92%) and dairy milk (50.90%). In probiotic *L. sporogenes* × *L. acidophilus* ($P_1 \times P_2$), maximum total sugar was found in buffalo milk (54.85%) followed by cattle milk (54.63%) and dairy milk (53.72%) presented in (Fig. 2).

The highest total sugar was found in $P_1 \times P_2$ (54.63%) followed by P_2 (54.35%), P_3 (53.92%), P_1 (53.13%) and the lowest total sugar was found in C (46.48%) in the shrikhand prepared using cattle milk and in the shrikhand prepared using buffalo milk, the highest total sugar was found in $P_1 \times P_2$ (54.85%) followed by P_1 (54.57%), P_2 (54.43%), P_3 (54.22%) and the lowest total sugar was found in C (48.02%). Similarly, in the shrikhand prepared using dairy milk, the highest total sugar was found in $P_1 \times P_2$ (53.72%) followed by P_2 (53.52%), P_1 (51.26%), P_3 (50.90%) and the lowest total sugar was found in C. In the present study, the highest total sugar was observed in $P_1 \times P_2$ (*Lactobacillus sporogenes* + *L. acidophilus*) followed by P_1 (*L. sporogenes*), P_2 (*L. acidophilus*) and P_3 (*L. rhamnosus*), the lowest total sugar was observed in control. This might be due to the blending of sugar with chakka, increased the total sugar simultaneously. The sugar slowed down the chemical changes due to fermentation. This is in conformation with the results reported by Boghra and Mathur (13), Navita et al. (14), Osman and Razig (12).

Residual Sugars (%)

The residual sugar (%) in control (C), maximum were found in buffalo milk (20.80%) followed by cattle milk (20.43%) and dairy milk (20.10%). In probiotic *L. sporogenes* (P_1), maximum residual sugars were found in buffalo milk (22.43%) followed by cattle milk (21.26%) and dairy milk (21.23%). In probiotic *L. acidophilus* (P_2), maximum residual sugars were found in buffalo milk (22.50%) followed by cattle milk (21.36%) and dairy milk (21.10%). In probiotic *L. rhamnosus* (P_3), maximum residual sugars was found in buffalo milk (22.36%) followed by cattle milk (21.31%) and dairy milk (21.11%). In probiotic *L. sporogenes* × *L. acidophilus* ($P_1 \times P_2$), maximum residual sugars was found in buffalo milk (22.63%) followed by cattle milk (22.59%) and dairy milk (22.33%) presented in (Fig. 3).

In shrikhand prepared by using cattle milk, the highest residual sugars was found in $P_1 \times P_2$ (22.59%) followed by P_2 (21.36%), P_3 (21.31%), P_1 (21.26%) and the lowest residual sugars was found in C (20.43%) and in the shrikhand prepared using buffalo milk, the highest residual sugars was found in $P_1 \times P_2$ (22.63%) followed by P_2 (22.50%), P_1 (22.43%), P_3 (22.36%) and the lowest residual sugars was found in C (20.80%). Similarly, in shrikhand prepared using dairy milk, the highest residual sugar was found in $P_1 \times P_2$ (22.33%) followed by P_1 (21.23%), P_3 (21.11%), P_2 (21.10%) and the lowest residual sugars was found in control (20.10%). The residual sugar is an final indicator of fermentation efficiency. In this experiment residual sugar concentration was highest in $P_1 \times P_2$ (*Lactoba-*

cillus sporogenes + *L. acidophilus*) treatment followed by P_2 (*L. acidophilus*), the lowest residual sugar concentration was observed in control. This may be due to its influence in sugar utilization by lactic acid bacteria. These result are similar to those of Karthikeyan et al. (15). Osman and Razig (12).

pH of Shrikhand

The pH of the shrikhand in control (C), maximum was found in buffalo milk (3.49) followed by cattle milk (3.48) and dairy milk (3.46). In probiotic *L. sporogenes* (P_1), maximum pH was found in buffalo milk (3.57) followed by cattle milk (3.52) and dairy milk (3.51). In probiotic *L. acidophilus* (P_2), maximum pH was found in buffalo milk (3.55) followed by cattle milk (3.53) and dairy milk (3.49). In probiotic *L. rhamnosus* (P_3), maximum pH was found in buffalo milk (3.52) followed by cattle milk (3.50) and dairy milk (3.46). In probiotic *L. sporogenes* × *L. acidophilus* ($P_1 \times P_2$), maximum pH was found in buffalo milk (3.69) followed by cattle milk (3.60) and dairy milk (3.58).

The shrikhand prepared by using cattle milk had the highest pH was found in $P_1 \times P_2$ (3.58) followed by P_2 (3.53), P_1 (3.51), P_3 (3.50) and the lowest pH was found in C (3.46) and the shrikhand prepared using buffalo milk had the highest pH in $P_1 \times P_2$ (3.69) followed by P_1 (3.57), P_2 (3.55), P_3 (3.52) and the lowest pH was found in PC (3.49). Similarly, in shrikhand prepared using dairy milk had the highest pH in $P_1 \times P_2$ (3.58) followed by P_1 (3.52), P_3 (3.50), P_2 (3.49) and the lowest pH was found in C (3.46). Those of similar results were obtained by Osman and Razig (12).

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