

Development and Quality Assessment of Probiotic Shrikhand Incorporated with Kiwi (*Actinidia Deliciosa*) Fruit Pulp

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Abstract: Milk preservation by fermentation is an old age technique. Shrikhand is a semi solid, sweetish-sour, whole milk product, prepared from lactic fermented curd. It is nutritionally dense fermented milk product. Its nutritional and therapeutic value can further be enhanced by incorporating filled milk, fruit pulp or probiotics. They are highly nutritious product because of increased vitamin content as selective cultures are being used in preparation of curd. The basic aim of the study was “Development of Probiotic Shrikhand incorporated with kiwi pulp.” Shrikhand was prepared using as per the standard method using 2% activated culture of *L.helvetics* and *S.thermophilus* and different levels of kiwi pulp 10%(T1), 20%(T2), 30%(T3), while Control sample T0 was prepared without addition of kiwi pulp and stored for 30 days at 4°C. Proximate composition of raw materials, Sensory attributes, Physico-chemical constituents and Microbiological analysis was analysed from prepared product and data collected from that were tabulated. Storage study of probiotic shrikhand (absence of coliforms) had good storage stability during 30 days of storage at refrigeration temperature (4°C). T1 (10%) supplementation of kiwi pulp to shrikhand was much preferred. Probiotic shrikhand could thus serve as good carrier of probiotic bacteria to improve our gut health. It also help in individuals having lactose intolerant. Hence, Shrikhand can provide therapeutic benefits to the consumer with longer shelf life.

Keywords: Shrikhand, Fresh Buffalo Milk, Lactic Acid Bacteria, Chakka, Probiotic, Kiwi Pulp

I. INTRODUCTION

Probiotic are live microorganism which when administered in adequate amounts confer a health benefit on the host. Lactic acid bacteria used as starters in production of dahi, shrikhand, mishti dahi, lassi, buttermilk and yoghurt. The lactic acid bacteria are naturally accepted as GRAS (generally regarded as safe) for human consumption. Widely used bacteria are of the genera *Lactobacillus* and have been therapeutically, to improve digestive process, prevent cancer, modulate immunity, and improve lactose intolerance.

Shrikhand is an most indigenous fermented & sweetened milk product having a typical pleasant sweet sour taste. Shrikhand is extensively used as a sweet dish after meals. Shrikhand has originated in western India. It is a very popular dessert. Shrikhand in Sanskrit means “Sandal”. Shrikhand the product might have been so named because of its flavour, the cooling effect and the colour, the qualities, which it shares with sandal. The fact is that this word is originated from the Sanskrit word “Shikharini” as mentioned in “Bhavaprakash”, the old classic. Shrikhand obtained from curd contains the most of the valuable constituents of milk like casein in a highly digestible form, minerals, fat and fat soluble vitamins and an appropriate amount of B complex vitamins, particularly riboflavin and folic acid. Shrikhand is ethnic fermented milk food assumes special importance due to its pleasant taste. And it is also very popular in Indian states i.e. Maharashtra, Gujarat, Karnataka and along in Rajasthan because of its high nutritive, therapeutic value, characteristic flavour and taste and palatable nature. Fermented milk and milk products occupy a place in satisfying nutritional requirements of human being since the time antiquity. Other varieties of shrikhand such as fruit shrikhand are also in great demand because of change in health habit of consumers and their economic status. Fermented milk products have been well recognized to have

therapeutic, anticholesterolemic, anticarcinogenic properties. Shrikhand is well known product for its better shelf-life because of prepared by lactic acid fermentation. Shrikhand is a whole milk product having semi-soft texture, sweetish sour taste. Shrikhand is served as special delicacy during festivals and ceremonial occasions. Consumption of shrikhand is effective in treatment of many diseases like diarrhea, acidity, gastro enteritis.

Kiwi (*Actinidia deliciosa*) is a highly nutritious unique fruit. It is very much valuable in terms of health. Kiwis are small fruits that pack a lot of flavour and plenty of health benefits. It is also full of nutrients like vitamin C, vitamin K, vitamin E, folate and potassium. They also have a lot of antioxidants and are a good source of fiber. A 2014 study found evidence that the bioactive substances in three kiwis a day can lower blood pressure more than one apple a day. Long term this may also mean a lowered risk for condition that can be caused by high blood pressure, like strokes or heart attacks.

II. MATERIALS AND METHOD

The present research work was an investigation on “Development of probiotic shrikhand incorporated with kiwi pulp”.

- **Locale of Study:** The experiment was accomplished in the laboratory of Food technology of parul institute of applied science vadodara, Gujarat, India.
- **Period of Experiment:** The experiment reported was conducted during the period from January 2021 to March 2021.
- **Procurement of Sample:** Fresh buffalo milk was procured from the local dairy, vadodara. Sugar and Ripened kiwi were purchased from local market of vadodara, Gujarat. The freeze dried culture of *Lactobacillus helveticus* and *Streptococcus thermophilus* was obtained from Sheth M.C. College of Dairy Science, Anand Agriculture university (AAU), Anand.

2.1 Experimental Design

The study was mainly divided in six phases.

Phase 1: Collection of materials

Phase 2: Sample preparation & raw material preparation

Starter culture, its maintenance and propagation

Phase 3: Development of probiotic shrikhand incorporated kiwi pulp

Phase 4: Sensory evaluation of the product

Phase 5: Determine physicochemical constituents of the product

Phase 6: To study shelf life of the product.

Phase 1:

Collection of Materials

Fully mature kiwi, powdered sugar were purchased from the local market and fresh buffalo milk having minimum 6% fat and 9% SNF was collected from the local dairy of Vadodara. And starter culture (*Lactobacillus helveticus* and *Streptococcus thermophilus*) was obtained from Sheth M.C. College of Dairy Science, Anand Agriculture University (AAU), Anand.

Phase 2:

Raw Material Preparation

Extraction of Kiwi Pulp

Fully mature fruit was selected. The fruits were washed with running tap water to remove dirt and dust. For making pulp it was peeled by using a knife and then cut into small pieces and then ground in a mixer till pulp is formed and pulp was filtered through a sieve to remove excessive seeds and obtained pulp was used to mix at different levels during shrikhand preparation.

Starter Culture, its Maintenance and Propagation

The activated pure culture of *Lactobacillus helveticus* and *Streptococcus thermophilus* was obtained from Sheth M.C. College of Dairy Science, Anand Agriculture university (AAU), Anand. The cultures were maintained separately in

sterilized skim milk test tubes. Then 1 ml of the cultures were transferred into 10 ml of 11% skim milk and incubated for 12-16 hr at 37°C and thereafter stored at refrigerator temperature (5°- 7°C). In order to keep this culture active, they were propagated once in a week (Shankar, 1975).

Phase 3: Development of probiotic shrikhand incorporated kiwi pulp

Procurement of all Ingredients

1. Milk:

Fresh buffalo milk is collected from the local dairy of vadodara.

2. Starter Culture

Lactobacillus helveticus and *Streptococcus thermophilus* procured from S.M. Seth College of Dairy Science, Anand.

3. Powdered Sugar

Powdered sugar was procured from local market of vadodara.

4. Kiwi

Kiwi was procured from local market of vadodara.

Standardization of Product

Treatments were prepared by using kiwi pulp at different levels in developed probiotic shrikhand.

Different ratios of kiwi pulp for preparation of Shrikhand

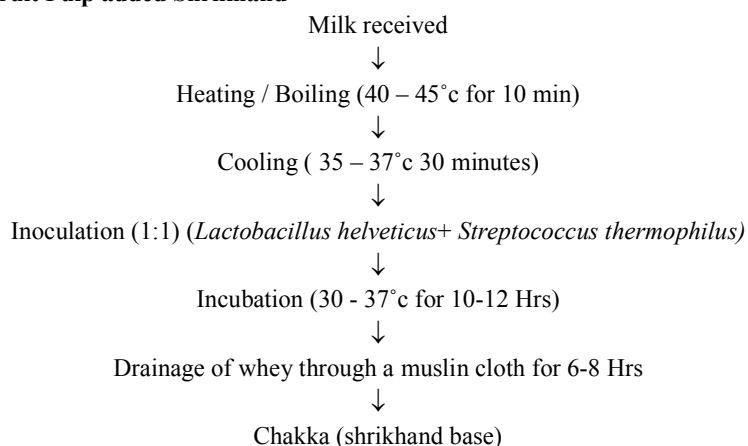
Ingredients	Control product	Experimental products		
		T0	T1	T2
Chakka (gm)	100	90	80	70
Powdered sugar	40	40	40	40
Kiwipulp (gm)	-	10	20	30

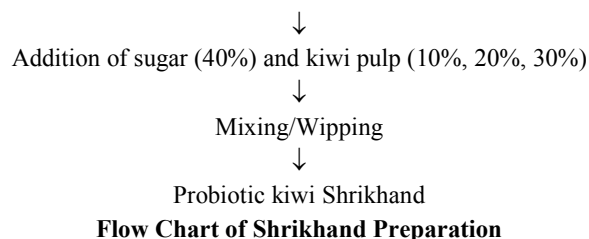
(40% Sugar is added by weight of chakka)

Preparation of Shrikhand

Fresh buffalo milk with 6% fat and 9% SNF was procured from the local dairy of vadodara. Milk was heated to 40-50°C for 10 min, it was then cooled to 35-37°C and inoculated by the starter culture *Lactobacillus helveticus* and *Streptococcus thermophilus* @ 1 : 1 ratio and then milk was incubated at 37°C for 10-12 hrs until a firm coagulum (Dahi) was formed. Coagulum was transferred to a muslin cloth and pressed in hoop for expulsion of whey for 6-8 hours. The semi-solid mass left after drainage of whey is called 'Chakka'. The chakka was used as the base material for preparation of shrikhand. This chakka was mixed with powdered sugar @ 40 Percent by weight of chakka and kiwi pulp in three different treatments 10%, 20%, 30% and the mixture was mixed uniformly. Control shrikhand was prepared without addition of kiwi pulp.

Preparation of Kiwi Fruit Pulp added Shrikhand





Phase 4:

Sensory Evaluation (9 point Hedonic Scale)

The product was served to a judges for sensory evaluation. And they were provided with nine points hedonic score card for evaluation as described in IS: 6273 (part-II), 1971^[10]. The probiotic shrikhand was evaluated by the judges for sensory attributes like colour, taste, aroma, flavour, appearance and overall acceptability.

Phase 5:

Determine Physicochemical Constituents of the Product

1. Ph (AOAC 2000)

The pH content was determined by (M.tronoic digital-255) pH meter

2. **Moisture:** The moisture content of shrikhand was determine by AOAC, 2000

3. **Total Solids:-**The total solids content of probiotic shrikhand was estimated by FSSAI manual, 2015

4. **Titratable acidity:** The titrable acidity of the probiotic shrikhand was determine by FSSAI MANUAL,2015

5. **Ash (AOAC 2000)**

6. **Fat (Acid hydrolysis)**

7. **Protein(AOAC 1990)**

8. **Lactose (FSSAI manual, 2015)**

9. **Total Fat(AOAC 1980)**

Fat extraction was determined by the method given by Soxhlet method according to (AOAC, 1980)

10. **Protein (AOAC 1980)**

Protein was estimated by the method given by khjeldhal method in (AOAC, 1980).4

Phase 6: To Evaluate Shelf Life of the Product

The probiotic shrikhand was packed in polystyrene cups and stored under refrigeration temperature (4°C). The packed probiotic shrikhand samples confirming to the different treatments (T0, T1, T2, T3) were studied for storage stability during a storage period of 30 days under refrigeration temperature (4°C)

Viability of Probiotic Bacteria in Shrikhand (AOAC, 2005)

Viability of probiotic bacteria was determined during the storage by serial dilution method. One gram of probiotic shrikhand samples were weighed and serially diluted upto 10¹⁰ dilutions were taken in duplicate into petri plates. The probiotic bacterial population was enumerated by pour plate technique using MRS media after incubation at 40°C for 48 hr.

Enumeration of Coliforms (AOAC, 2005)

The second, third and fourth dilutions of probiotic shrikhand samples were prepared,has been used for enumeration of coliforms. The 1 ml from 10², 10³, 10⁴ dilution were taken in duplicate into petri plates and the violet red bile agar was added and mixed well. The plates were allowed to solidify. These plates were incubated at 37°C for 1-2 days.

Enumeration of Yeast and Mould (AOAC, 2005)

The 10⁸, 10⁹,10¹⁰ dilutions of probiotic shrikhand samples were taken in duplicate into petri plates. It was then pour plated using yeast PDA (Potatoes dextrose agar) and then incubates at 22-25°C room temperature for 3-5 days

III. RESULTS AND DISCUSSION

3.1 Organoleptic Evaluation of Probiotic Shrikhand

Sr. No.	Sample Code	Colour	Aroma	Taste	Flavour	Appearance	Overall Acceptability
1	T0	6	5.4	3.2	3.6	5	4.64
2	T1	8.6	8.2	9	9	8.4	8.64
3	T2	8.4	8	6.6	5	5.6	6.72
4	T3	5.2	4.4	4.8	4.4	4.8	4.72

Table: Organoleptic analysis of prepared probiotic shrikhand

Value of sensory attributes of prepared product scored by 5 members using 9 point Hedonic scale. Shrikhand was tested for colour, Taste appearance, Flavour, aroma and overall acceptability.

- **Colour:** There was significant difference in color score observed in different treatment combination. Maximum colour score of 8.6 was found in the treatment T1 followed by sample T0, T2, T3 the score were 6, 8.6, 8.4, 5.2, was observed.
- **Aroma:** There was significant difference in aroma score observed in different treatment combination. Maximum aroma score of 8.2 was found in the treatment T1 followed by sample T0, T2, T3 the score were 5.4, 8, 4.4 was observed.
- **Taste:** There was significant difference in taste score observed in different treatment combination. Maximum taste score of 9 was found in the treatment T1 followed by sample T0, T2, T3 the score were 3.2, 6.6, 4.8 was observed.
- **Flavour:** There was significant difference in flavour score observed in different treatment combination. Maximum flavour score of 9 was found in the treatment T1 followed by sample T0, T2, T3 the score was 3.6, 5, 4.8 was observed.
- **Appearance:** There was significant difference in appearance score observed in different treatment combination. Maximum appearance score of 8.4 was found in the treatment T1 followed by sample T0, T2, T3 the score was 5, 5.6, 4.8 was observed.
- **Overall acceptability:** In sensory attributes maximum overall acceptability scores 8.64 was found in treatment T1 followed by sample T2, T3, T0 the score was 6.72, 4.72, 4.64 was observed.

3.2 Physio-Chemical Analysis

Treatment	Acidity	P ^H	Moisture	Total solid	Ash	Fat	Protein	Lactose
T0	1.83	3.41	45.12	58.54	1.21	8.93	7.98	8.5
T1	2.02	3.63	48.73	56.69	1.18	8.62	7.86	7.2
T2	2.2	3.67	50.07	53.83	1.15	8.25	6.93	6.5
T3	2.43	3.69	43.46	51.72	1.09	7.76	6.80	6.2
S/NS	S	S	S	S	S	S	S	S

(S= significant, NS= non-significant)

- **Acidity:** The average acidity per cent of finished product found to be 1.83, 2.02, 2.2 and 2.43 per cent for treatment T0, T1, T2 and T3 respectively. The acidity content of developed product was increased as increased in concentration of kiwi fruit pulp per cent acidity in prepared probiotic shrikhand. Shrikhand prepared with 30 per cent kiwi pulp showed maximum Titrable acidity percentage (2.43) whereas the least found in control (1.83).
- **p^H:** The P^H content in the finished product as found to be 3.41, 3.63, 3.67 and 3.69 per cent for treatment T0, T1, T2, and T3 respectively. The higher P^H found to be 3.41(T0) and lower P^H 3.69 (T3). It is observed that P^H increases due to increase in kiwi fruit pulp

A. Moisture

There was a corresponding increase in moisture content in all the shrikhand samples. Moisture level was higher in (T3) 30% kiwi pulp but lower in (T0) without kiwi pulp. This might due to higher water holding capacity of interactive mixture of kiwi fruit and milk solids. The moisture content of T1(45.12%), T2(48.73%), T3(50.07%) compared to control T0(43.46) was recorded. Moisture level is higher in 30% kiwi pulp but lower in without kiwi pulp.

B. Total Solid

Total solid content of shrikhand decreased with an increase in the level of kiwi pulp. The maximum total solid content (58.54%) was noticed in shrikhand without kiwi pulp. The simultaneous decrease from 58 to 51 may be due to lower content in kiwi pulp. Moreover, it can be depicted from the results that the addition of 10% of kiwi pulp got maximum total solid percentage (56.69%) whereas the minimum total solid percent (51.72) was observed in shrikhand with 30% kiwi pulp i.e. T3

C. Ash

The average ash content of finished product was 1.21, 1.18, 1.15 and 1.09 in treatment T0, T1, T2 and T3 respectively. It was also observed that the lowest ash content was in T3 and highest was found in control sample (T0). Increase in the level of kiwi fruit pulp resulted in significant decrease in ash content of shrikhand. The highest ash content (1.21%) was observed in shrikhand prepared without pulp i.e (T0), whereas the lowest percent (1.09%) found in shrikhand with 30 per cent kiwi fruit pulp (T3). It is observed that decreased the ash content as the kiwi fruit pulp was increased.

D. Fat

The fat content ranged between 7.76 to 8.93 per cent. shrikhand with kiwi pulp contained significantly lower amount of fat than the control shrikhand(T0).the highest fat content was found 8.93 percent in control shrikhand. Among all the samples blended with kiwi pulp maximum fat content 8.62 percent in case of 10% level of kiwi pulp, followed by 8.25 and 7.76 percent in case of 20 percent and 30 percent level of kiwi pulp, respectively. As loss of moisture decreases fat, increase in moisture increase fat, vice versa.

E. Protein

There was a gradual decrease in protein content of shrikhand with increase in level of kiwi pulp. The Maximum protein content was recorded for control treatment (T0) i.e 7.98 per cent and the minimum protein content was recorded for treatment (T3) i.e 6.80 per cent.

F. Lactose

The average lactose content of a finished product was 8.5, 7.2, 6.5 and 6.3 in treatment T0, T1, T2 and T3 respectively. It is observed that there was a decrease in level of lactose. The maximum lactose content was noted in treatment T1 (8.5%) whereas the lowest lactose content was found in treatment T3 (6.3%) .

3.3 Microbiological Analysis

Changes in Microbial Activity during Storage of Probiotic Shrikhand

Microbial quality	0 day				15 day				30 day			
	C	E			C	E			C	E		
	-	10 ⁸	10 ⁹	10 ¹⁰	-	10 ⁸	10 ⁹	10 ¹⁰	-	10 ⁸	10 ⁹	10 ¹⁰
Viability	-	UC	-	-	-	UC	-	-	-	-	UC	UC
Yeast and mould count	-	10 ⁴	10 ⁵	10 ⁶	-	10 ⁴	10 ⁵	10 ⁶	-	10 ⁴	10 ⁵	10 ⁶
	-	-	-	-	-	1× 10 ⁶ CfU/gm	-	-	-	-	UC	-
Coliform count	-	10 ²	10 ³	10 ⁴	-	10 ²	10 ³	10 ⁴	-	10 ²	10 ³	10 ⁴
	-	-	-	-	-	-	-	-	-	-	-	-

*C- control, *E-experimental *UC- uncountable, ‘-’ = Nil (cfu- colony forming unit)

A. Viability

The viability count of *Lactobacillus helveticus* and *Streptococcus thermophilus* was present at the 0 day of storage. Cluster of colony was observed in experimental samples T1 (10⁸dilution) which was uncountable due to the enormous cluster .After 15 and 30 days of storage it remained same uncountable in T3 (10⁹ and 10¹⁰ dilution) and sceptical to identify and to count, hence it was a fallible result.

B. Yeast and Mould Count

The yeast and mould growth in probiotic enriched shrikhand was analyzed at the regular intervals *viz.*, 10th, 20th and 30th days of storage. Initially there was no yeast and mould growth in probiotic shrikhand thus, yeast and mould were absent in fresh shrikhand. Then on 15th day of storage in one experimental (T2 -4th dilution) sample there was only a one colony was observed on plate and after that at 30th day (T2-5th dilution) of storage uncountable growth were seen on plate.

C. Coliform Count

In each treatment of prepared shrikhand the coliform count was nil which indicates that hygienic conditions adapted during manufacturing as well as the sanitation was carried out which was adequate.

IV. CONCLUSION

From the present investigation it can be stated that kiwi pulp can be very well utilized by blending it in different ratios with buffalo milk containing 6% fat, 1:1 starter culture of *L.helveticus* and *S.thermophilus* strain. The blend of treatment T1 was more acceptable than other experimental treatments T2 and T3 due to higher overall acceptability score. It was observed that as the increasing level of kiwi fruit pulp, there was also increased in P^H, Moisture, Acidity and decreased in fat, protein, total solid and ash content of kiwi fruit pulp added probiotic shrikhand. It concluded that value added shrikhand with its good quality and more acceptability can be prepared by the addition of kiwi fruit pulp. Sample T1 containing 10% kiwi fruit pulp was found most acceptable in the terms of sensory as well as physiochemical scores whereas treatment containing 20% and 30% kiwi fruit pulp also obtained satisfactory results as they were within the acceptable limit. shrikhand can be stored for 30 days due to absence of coliforms and yeast and mould was also lower, and it can be recommended as health food for patients due to its lower fat content and being a probiotic such type of shrikhand will be beneficial to health conscious people.

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