Studies on storage stability of probiotic Shrikhand obtained from safflower (Carthamus tinctorius)-blended milk

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Abstract: Shrikhand is a semi solid, sweetish-sour, whole milk product, prepared from lactic fermented curd. The curd (dahi) is partially strained through a muslin cloth to remove the whey to yield Chakka. Sugar, flavour, colour and dry fruits/condiments are mixed into Chakka to form a soft homogenous mass known as Shrikhand. It is a nutritionally dense fermented milk product. Its nutritional and therapeutic value can further be enhanced by incorporating filled milk, fruit pulp or probiotics. Safflower seed is rich in essential fatty acid (78% linoleic acid). Safflower milk blended probiotic Shrikhand was developed by using safflower milk, lactic culture, probiotic culture, sugar, powdered safflower petals (colorant) and mango flavour. Among the various levels of safflower milk-cow milk (CM) blends, lactic cultures and probiotic cultures tried in preparation of probiotic Dahi, the blends of 30:70 safflower milk-CM, scored the highest. Chakka prepared from optimized probiotic Dahi used for Shrikhand preparation using 40% sugar, 0.1% colour and 500 ppm mango flavour was adjudged the best. Modified Shrikhand showed 12.47, 9.40, 40.70, 63.77 and 1.09% of fat, protein, carbohydrate, total solids and titratable acidity (% LA), respectively. Storage studies at refrigeration temperature (5±1°C) revealed that the product retained acceptability for a period of 21 days.

Keywords: Probiotics, Polyunsaturated Fatty Acids (PUFA), Shrikhand, Safflower Milk

Introduction

India has a very rich variety of fermented foods prepared from milk, cereals, pulses vegetables, fruits and fish. Milk and milk products like Dahi, buttermilk, Lassi, Shrikhand. The Shrikhand is a traditional indigenous fermented semi soft, sweetened whole milk product prepared using Chakka (strained Dahi). Dairy products are likely to remain important dietary components because of their nutritional value, flavor and texture. There will continue to be a demand for traditional, high quality dairy products, despite increasing competition from non-dairy based products. Trend of value-added and health promoting milk-based products is increasing swiftly. In view of this the present investigation was undertaken to evaluate suitability of incorporating safflower solids in the Shrikhand.

Safflower (Carthamus tinctorius L.) is an oilseed crop, a member of the family ‘Compositae’ or ‘Asteraceae’. Safflower has been grown in India since time immemorial. It is mentioned as kusumba in ancient scriptures. It has been grown in India for the orange-red dye (Carthamin) and yellow dye (Carthamidin) extracted from its brilliantly colored flowers and for its quality oil, rich in polyunsaturated fatty acids (PUFA) like linoleic acid (78%). Flowers of the Safflower are known to have many medicinal properties for curing several chronic diseases and are widely used in Chinese herbal preparations (Li and Mundel, 1996). The tender leaves and shoots of safflower are used as pot herb and salad. These are rich in vitamin A, iron, phosphorus, and calcium. Bundles of young plants are commonly sold as a green vegetable in markets in India and some neighboring countries (Nimbkar, 2002; Sarode et al. 2007). Safflower can be grazed or stored as hay or silage. Safflower forage is palatable, and its feed value and yields are similar to or better than those for oats or alfalfa.

Materials and methods

Bacterial cultures

The mixed lactic cultures and probiotic cultures (Lactobacillus acidophilus LA-5 and Bifidobacterium bifidum BB-12) were obtained from the Deptt. of Dairy Microbiology. Whole milk was procured from the Student Experimental Dairy Plant (SEDP), Dairy
Physico-chemical characteristics of safflower milk-CM blended

Results and discussion

R-software.

of variance was used for the storage study by using a statistical

incorporated probiotic

significant difference (P < 0.05) in the total solid contents among all the three products.

Effect of storage on soluble nitrogen of optimized probiotic Shrikhand

The experimental and the control Shrikhand samples were stored at refrigeration temperature at (5±1°C) and analysed for the attributes viz; soluble nitrogen, FFA and titratable acidity, once in week still visual defects were noticed.

Effect of storage on soluble nitrogen of optimized probiotic Shrikhand

The proteolytic activity of the viable bacteria present in control and Shrikhand samples represents the soluble nitrogen content. Results pertaining to impact of storage on sensory attributes of optimized probiotic shrikhand are presented in Table 2. The initial (day first) soluble nitrogen content was 0.50, 0.54 and 0.96% for control, PSWSM and probiotic PSSM, respectively. The soluble nitrogen percentage increased during storage period. At the end of storage period i.e. 28th day, control Shrikhand, probiotic shrikhand samples were stored soluble nitrogen content of 1.19, 1.25 and 1.36%, respectively. The soluble nitrogen content was significantly different in three products throughout the storage period. The values of soluble nitrogen give a clear indication of proteolytic activity of viable bacteria in probiotic Shrikhand. As the storage period advanced, production of lactic acid increased, which can inhibit the proteolytic activity of viable bacteria (Yadav et al. 2007).

Effect of storage on FFA content of optimized probiotic shrikhand

The lipolytic activity of the viable bacteria present in control and optimized probiotic shrikhand samples represents the free fatty acid content (% oleic acid). The effect of storage at refrigeration temperature (5±1°C) on FFA content is depicted in Table 2. The FFA percentage increased during storage period. The FFA content at day 21st was 0.38, 0.39 and 0.49 % for control, probiotic shrikhand without safflower milk and PSSM, respectively. At the end of storage period i.e. 28th day, the control shrikhand, probiotic Shrikhand without safflower milk and PSSM showed FFA content of 0.42, 0.42 and 0.55 % oleic acid, respectively. Statistical analysis of the data revealed that PSSM contained significantly
higher FFA content as compared with the control *Shrikhand* without safflower throughout the storage period.

**Effect of storage on titratable acidity of optimized probiotic shrikhand**

The effect of storage at refrigeration temperature (5±1°C) on titratable acidity is shown in Table 2. The titratable acidity at day first was found to be 1.19, 1.14 and 1.09 (% LA) for control, PSWSM and PSSM, respectively. It was gradually increased during storage period. At the end of 28th day, the control *Shrikhand*, PSWSM and PSSM showed a titratable acidity of 1.44, 1.41 and 1.42 (% LA), respectively. The rate of increase in acidity during the storage period in PSSM was slower as compared to the control and PSWSM. Statistical analysis revealed that the difference between the titratable acidities of PSSM and the control during the storage period was nonsignificant throughout the storage period.

**Effect of storage on the microbial quality of the optimized probiotic shrikhand**

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**Fig. 1** Flow chart for production of *Shrikhand*
For the safety of consumer, yeast and mold and coliform counts in experimental Shrikhand was estimated. The control, PSWSM and PSSM were subjected to microbial analysis. The data on the influence of storage on the microbial quality of samples expressed as log_{10} cfu/g during 0 to 28 days of storage at (5±1°C) at an interval of 7 days revealed that the yeast and mold count was nil in all the samples up to 21st day of storage. A non significant difference was recorded on 28th day in the yeast and mould counts.

Effect of storage on coliform counts of the optimized probiotic Shrikhand

The coliform count was nil in control Shrikhand, PSWSM and PSSM up to 21st day of storage at (5±1°C). The counts on 28th day of storage in the control, PSWSM and PSSM showed coliform counts of 1.84, 1.47 and 2.25 cfu/g, respectively. The PSSM showed highest coliform counts compared to other samples on 28th day that made the product unfit for consumption. The experimental probiotic shrikhand met the coliform requirement of FSSAI (2006) standards i.e. max. 50/g up to the 21st day of storage. Hence the PSSM stored at 5±1°C is safe for consumption only up to 21 days. Similar results were noticed for control non-probiotic Shrikhand stored for 24 days and carrot enriched probiotic Shrikhand stored for 30 days at refrigeration condition prepared by using probiotic culture (Patil, 2014; Srinivasa, 2008).

Table 1 Physicochemical characteristics of the optimized safflower milk blended probiotic Shrikhand

| Type of samples | Fat (%)  | Protein (%) | Total sugar (%) | Titratable acidity (% LA) | TS (%)  |
|-----------------|----------|-------------|-----------------|--------------------------|---------|
| Control         | 11.13a   | 9.82a       | 40.72a          | 1.19a                    | 62.92a  |
| PS              | 11.26a   | 9.73a       | 40.70a          | 1.14a                    | 62.60a  |
| PSSM            | 12.47b   | 9.40b       | 40.70a          | 1.09b                    | 63.77c  |
| CD (P<0.05)     | 0.30     | 1.41        | 0.08            | 1.74                     | 0.20    |

All the values are averages of three replications; Similar superscripts indicate non-significant differences at the corresponding critical differences.

Table 2 Effect of storage at (5±1°C) on Soluble nitrogen (%), Free fatty acid (%oleic) content and Titratable acidity (%LA) of the optimized probiotic Shrikhand

| Type of sample | Parameters                  | 0    | 7    | 14   | 21   | 28   |
|----------------|-----------------------------|------|------|------|------|------|
| Control        | Soluble nitrogen (%)        | 0.50a| 0.54a| 0.79a| 0.92a| 1.19a|
|                | Free fatty acid (% oleic)   | 0.27a| 0.31a| 0.35a| 0.38a| 0.42a|
|                | Titratable acidity (% LA)   | 1.19a| 1.25a| 1.31a| 1.38a| 1.44a|
| PSWSM          | Soluble nitrogen (%)        | 0.53a| 0.58a| 0.81a| 1.00b| 1.25b|
|                | Free fatty acid (% oleic)   | 0.27a| 0.32a| 0.36a| 0.39a| 0.42a|
|                | Titratable acidity (% LA)   | 1.14a| 1.19a| 1.25a| 1.35a| 1.41b|
| PSSM           | Soluble nitrogen (%)        | 0.96c| 1.13b| 1.24b| 1.26b| 1.36b|
|                | Free fatty acid (% oleic)   | 0.31b| 0.37b| 0.42b| 0.49b| 0.55b|
|                | Titratable acidity (% LA)   | 1.09c| 1.11b| 1.27b| 1.37c| 1.42b|
| CD (P<0.05)    | Soluble nitrogen (%)        | 0.43 | 0.32 | 0.34 | 0.30 | 0.15 |
|                | Free fatty acid (% oleic)   | 0.01 | 0.03 | 0.04 | 0.08 | 0.11 |
|                | Titratable acidity (% LA)   | 0.05 | 0.13 | 0.03 | 0.02 | 0.02 |

All the values are averages of three replications; Similar superscripts indicate non-significant differences at the corresponding critical differences.

Table 3 Effect of safflower milk on the growth of Bifidobacterium bifidum in Shrikhand during the storage (5±1°C)

| Storage period (days) | PS | PSSM |
|-----------------------|----|------|
|                       | Viable counts (log cfu/g) |       |
| 0                     | 8.99 | 9.08 |
| 7                     | 8.98 | 9.07 |
| 14                    | 7.79 | 7.96 |
| 21                    | 7.70 | 7.80 |
| 28                    | 5.51 | 5.63 |

For the safety of consumer, yeast and mold and coliform counts in experimental Shrikhand was estimated. The control, PSWSM and PSSM were subjected to microbial analysis. The data on the influence of storage on the microbial quality of samples expressed as log_{10} cfu/g during 0 to 28 days of storage at (5±1°C) at an interval of 7 days revealed that the yeast and mold count was nil in all the samples up to 21st day of storage. A non significant difference was recorded on 28th day in the yeast and mould counts.

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Effect of safflower milk on the viability of Bif. bifidum in PSSM during the storage (5±1°C)
The effect of storage on the growth of *Bif. bifidum* in PSWSM and PSSM during storage period from 0 to 28 days at (5±1°C) at an interval of 7 days is presented in Table 3.

The counts on day 0, 7, 14, 21 and 28 were 8.99, 8.98, 7.79, 7.70 and 5.51, respectively for PSWSM whereas in PSSM the counts were 9.08, 9.07, 7.96, 7.80 and 5.63, respectively. As the storage period advanced, the viable counts decreased. It was observed that safflower milk exhibited slightly positive effect on the growth of *Bif. bifidum* counts. During the storage the counts decreased gradually in both types of *Shrikhand*. However, the PSSM retained the higher count of *Bif. bifidum* compared to the PSWSM. The counts showed declines during storage from 0 to 28 days in both types of *Shrikhand*. Earlier workers (Dave and Shah, 1998) reported a significant increase in the acidity of probiotic yoghurt in experimental samples as the storage period advanced.

The results obtained in the present investigation are comparable to those reported earlier (Krishana et al. 2011) where *Lassi* with added probiotic bacteria stored at 4±1°C for a period of 15 days showed a 2 log cycle reduction of viable counts of *L. acidophilus* and *Bif. bifidum* at the end of the storage period. Acid production of lactic acid bacteria after the incubation decreased the viability of probiotic bacteria especially *Bifidobacterium* ssp (Ishibashi and Shimamura, 1993).

Reduction in viability of probiotic cultures (*L. acidophilus* and *B. bifidum*) in WPC enriched shrikhand stored at room temperature was reported earlier (Harrigan, 1998). However, in the present study, the viable counts of *Bif. bifidum* at 21st days of storage at 5±1°C, observed in experimental shrikhand was log 7.80 cfu/g, minimum of 10⁶ cells of probiotics are required in a product to extend health benefits as many authors have suggested. The safflower milk incorporation in the preparation of probiotic shrikhand might have supported the growth of *Bif. bifidum* proving its prebiotic effect.

**Conclusions**

It is concluded from the present investigation that formulated PSSM is nutritionally dense, which can be promoted for regular consumption. Synergy of dairy and agro products can bring down overall cost of production which can impart more benefits to the farmers as well as the lower. Formulated probiotic shrikhand has 21 days shelf-life at refrigeration temperature (5±1°C) which can be improved by using advanced packaging techniques.

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