RESEARCH ARTICLE

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

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Received: 21 September 2018 / Accepted: 15 January 2019 / Published online: 18 April 2019
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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 ($5\pm1^{\circ}\text{C}$) revealed that the product retained acceptability for a period of 21 days.

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

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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

Science College, Karnataka Veterinary and Fishery Sciences University (KVAFSU), Hebbal, Bengaluru (India) and the safflower seeds and safflower flower petals of *Sharda* variety were brought from the Parbhani district of Maharashtra state. The colourless artificial mango flavour was procured from Down Foods and Flavours, Bengaluru.

Determination of chemical composition of safflower milk

The compositional parameters *viz.* moisture, protein, fat, crude fiber, total carbohydrate, minerals and total solids were determined as per the method (AOAC, 2000).

Physico-chemical analysis of optimized probiotic *dahi* and *Shrikhand*

The product was prepared as shown in the flow diagram (Fig. 1). The physico-chemical analysis *viz.*, fat, titratable acidity, protein, moisture, total solids of safflower milk blended optimized probiotic *dahi* and *Shrikhand* were determined as per the method described earlier (Indian Standards, 1980; Indian Standards, 1981). However, the pH was monitored by a digital meter and syneresis was determined by the method mentioned earlier (Gatson et al. 2007).

Microbial studies of the *Shrikhand*

Storage studies were conducted at refrigeration temperature ($5\pm1^{\circ}\text{C}$) and every week the respective sets of the products were tested for its probiotic counts. To enumerate Bifidobacterium counts anaerobic condition was maintained (Harrigan 1998), coliform, yeast and mold counts (Indian Standards, 1980). The initial and final microbial counts were estimated. Samples were diluted to 10^3 for coliform, yeast and mold counts whereas 10^6 for probiotic count. The media used for enumeration of various microbial counts (Probiotic counts: (a) *L. acidophilus*-MRS agar and (b) Bifidobacterium counts- Bifidus agar; Coliform counts- MacConkey's agar and Yeast and Mould counts-Potato Dextrose Agar) were as per the recommendations made earlier (HiMedia, 1998). To enumerate the bifidobacterial counts in the product, the product was packed in polythene cups, which were stored under anaerobic condition during the storage studies.

Statistical analysis

Analysis of variance technique (F-test) was employed for the nutrients composition and sensory characteristics to test the significant difference ($P\leq 0.05$) between control and safflower milk incorporated probiotic *Shrikhand* in the study. One-way analysis of variance was used for the storage study by using a statistical R-software.

Results and discussion

Physico-chemical characteristics of safflower milk-CM blended probiotic *Shrikhand*

The results pertaining to impact of safflower milk blending with CM in the preparation of probiotic *Shrikhand* are presented in (Table 1). Probiotic *Shrikhand* prepared from the *Dahi* obtained from safflower-blended milk (PSSM) showed higher fat and lower protein content compared to the control and probiotic *Shrikhand* without safflower milk (PS). All the three samples of *Shrikhand* were found to contain same total sugar content where as the PSSM showed low titratable acidity compared to remaining two types of *Shrikhand* samples. The total solid content was found to be 62.92, 62.60 and 63.77, respectively for all the three samples. Results reveal that there was a significant difference ($P\leq 0.05$) in the total solid contents among all the three products.

Effect of storage on various chemical attributes of the optimized safflower milk blended probiotic *Shrikhand*

The experimental and the control *Shrikhand* samples were stored at refrigeration temperature at ($5\pm1^{\circ}\text{C}$) 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*, PSWSM and PSSM showed 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 ($5\pm1^{\circ}\text{C}$) 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

Fig. 1 Flow chart for production of *Shrikhand*

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 ($5\pm 1^{\circ}\text{C}$) 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

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

Type of samples	Fat (%)	Protein (%)	Total sugar (%)	Titrateable acidity(% LA)	TS (%)
Control	11.13 ^a	9.82 ^a	40.72 ^a	1.19 ^a	62.92 ^a
PS	11.26 ^a	9.73 ^a	40.70 ^a	1.14 ^a	62.60 ^b
PSSM	12.47 ^b	9.40 ^a	40.70 ^a	1.09 ^a	63.77 ^c
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 Titrateable acidity (%LA) of the optimized probiotic *Shrikhand*

Type of sample	Parameters	Storage stability (days)				
		0	7	14	21	28
Control	Soluble nitrogen (%)	0.50 ^a	0.54 ^a	0.79 ^a	0.92 ^a	1.19 ^a
	Free fatty acid (% oleic)	0.27 ^a	0.31 ^a	0.35 ^a	0.38 ^a	0.42 ^a
	Titrateable acidity (% LA)	1.19 ^a	1.25 ^a	1.31 ^a	1.38 ^a	1.44 ^a
PSWSM	Soluble nitrogen (%)	0.53 ^a	0.58 ^a	0.81 ^a	1.00 ^{ab}	1.25 ^{ab}
	Free fatty acid (% oleic)	0.27 ^a	0.32 ^a	0.36 ^a	0.39 ^a	0.42 ^a
	Titrateable acidity (% LA)	1.14 ^b	1.19 ^a	1.25 ^b	1.35 ^b	1.41 ^b
PSSM	Soluble nitrogen (%)	0.96 ^b	1.13 ^b	1.24 ^b	1.26 ^b	1.36 ^b
	Free fatty acid (% oleic)	0.31 ^b	0.37 ^b	0.42 ^b	0.49 ^b	0.55 ^b
	Titrateable acidity (% LA)	1.09 ^c	1.11 ^b	1.27 ^b	1.37 ^c	1.42 ^b
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
	Titrateable 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 Viable counts (log cfu/g)	PSSM
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 log₁₀ 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).

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 ($5\pm 1^\circ\text{C}$) 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 $4\pm 1^\circ\text{C}$ for a period of 15 days showed 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 $5\pm 1^\circ\text{C}$, observed in experimental shrikhand was $\log 7.80$ cfu/g, minimum of 10^6 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 ($5\pm 1^\circ\text{C}$) which can be improved by using advanced packaging techniques.

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