

Development of Value Added Dahi with Safflower (*Carthamus Tinctorius*) Milk and Probiotics

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Abstract

Safflower (*Carthamus tinctorius*) milk was prepared by grinding soaked safflower seeds with water at 1:5 ratio. Compositional analysis showed 89.90% moisture, 5.48% fat, 1.77% protein, 2.36% carbohydrate, 0.45% ash and 0.04% crude fibre. Safflower milk blended probiotic dahi was developed by using safflower milk-cow milk blends of 20:80, 30:70 and 40:60 ratio, lactic cultures at 1, 1.5 and 2 percent, probiotic cultures viz., *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12 at 3, 5 and mixed cultures of these at 3 and 5 percent levels. Control probiotic dahi was prepared without incorporation of safflower milk. Among the various levels of safflower milk-cow milk blends, lactic cultures and probiotic cultures tried in probiotic dahi, the blends of 30:70 safflower milk-cow milk, 1.5% lactic culture and 5% *Bifidobacterium bifidum* respectively, scored maximum sensory scores. Safflower milk blended probiotic dahi showed 12.45, 3.73, 8.72, 2.24, 3.12, 0.60 and 0.82 percent of total solids fat, SNF, carbohydrate, protein, ash and titratable acidity (% LA) respectively, as against the control dahi (12.41, 3.63, 8.78, 2.12, 3.50, 0.65 and 0.80 percent).

Keywords: Lactic cultures; *Lactobacillus acidophilus*; *Bifidobacterium bifidum*; Acidity; Dahi; Optimization; Safflower Milk; Physicochemical Characteristics

Abbreviations

GRAS: Generally Recognized As Safe; SNF: Solid Not Fat; AOAC: Association of Official Analytical Chemists; IS: Indian Standards; PUFA: Poly Unsaturated Fatty Acids

Introduction

Dahi is a popular fermented dairy product of Indian subcontinent and is known for its refreshing taste, palatability and therapeutic values. Dahi resembles Leben in Iran, Jugart in Turkey, Roba in Iraq, Mazun in America, Villi in Finland and Shosim in Nepal [1]. Chakka, the base material used for the preparation of shrikhand is obtained from dahi by removal of whey from it. The Dahi bacteria especially lactic acid bacteria have been accepted as GRAS (Generally Recognized as Safe) for human consumption [2].

Consumption of dahi provides several health benefits to human health. But, dahi bacteria may not have probiotic potentials and may not be as good as it is labelled. Administration of probiotic bacteria can be useful to treat intestinal disorders. [3] However, in spite of remarkable increase in the production, the milk and milk products are out of reach to the vulnerable groups of weaker section of society due to high cost. This calls for development of low cost and healthy substitute for milk. Some health conscious people are skeptical in consuming milk products as these contain satu-

rated fatty acids and cholesterol. Safflower (*Carthamus tinctorius* L) is an oil seed crop rich in PUFA (Linoleic acid 78%) which is considerably low in bovine milk [4]. The aqueous extract of the safflower seeds - safflower milk has compatibility with bovine milk.

Value addition of dahi is a tool to increase its nutritional and functional properties. Hence, the present study was carried out to develop a probiotic value added dahi using the blends of safflower milk - cow milk and to evaluate their sensory and physicochemical characteristics.

Materials and Methods

Fresh whole milk procured from Students Experimental Dairy Plant (SEDP), Dairy Science College, Karnataka Veterinary Animal and Fishery Sciences University, Hebbal, Bengaluru, was standardized to 3.5% milk fat and 8.5% SNF using Skim milk powder and cream. The milk was preheated to 60 - 65 C, then homogenized (REMI) at 2000 psi at first stage, 500 psi at second stage and heat treated to 90 C for 10 min followed by cooling to 30 ± 1 C.

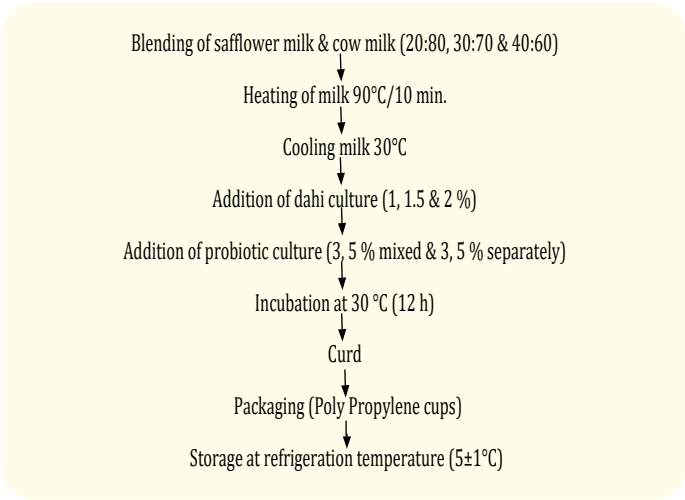
The commercial Nandini dahi cultures and probiotic cultures (*Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12) were obtained from Department of Dairy Microbiology, Dairy Science College, Bengaluru.

Fresh safflower seeds of Tara variety were procured from Parbhani district of Maharashtra state. Safflower milk was prepared as per [5] method.

Optimization of Dahi:

The safflower milk and cow milk blend was used to optimize levels of lactic culture and probiotic culture for the preparation of dahi. Nandini dahi culture (1%, 1.5% and 2%), probiotic cultures *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* BB-12 (3% and 5%) and mix of these probiotics at 1:1 ratio were used separately in optimized safflower milk blended with cow milk. Control probiotic dahi was prepared with the standardised cow milk without safflower milk.

Flow chart for the production of probiotic dahi



Chemical Composition of Safflower Milk

The compositional parameters like moisture, protein, fat, crude

fibre, total carbohydrate, minerals and total solids were determined as per the methods described in [6].

Sensory Attributes of Safflower Milk Cow Milk Blended Dahi

The prepared dahi samples were served to a panel of 5 judges to evaluate sensory attributes of product. Sensory evolution was done by using 9-point hedonic scale.

Physicochemical Characteristics of Safflower Milk, Cow Milk and Optimized Probiotic Dahi

The physico-chemical analysis of safflower milk blended optimized probiotic dahi viz; fat, titratable acidity, protein, moisture, total solids were determined as per the methods described in [7].

The physico-chemical analysis of safflower milk blended optimized probiotic dahi viz; fat, titratable acidity, protein, moisture, total solids were determined as per the methods described in [7]. pH of the dahi was determined by using digital pH meter (Chemi Line, India).

Statistical analysis of the results obtained was carried out using SPSS v 1.6.

Results and Discussion

Physicochemical Characteristics of Cow Milk and Safflower Milk

Safflower milk was greyish white in colour, bland in taste, while cow milk was pale yellow in colour with very mild sweetness. The cow milk was found to contain 87.59, 3.63, 3.50, 4.12, 0.65 and 0.12 percent of moisture, fat, protein, lactose, ash and lactic acidity (% lactic acid) respectively (Table 1). The safflower milk showed 89.90, 5.48, 1.77, 2.36, 0.45, 0.03 and 0.04 percent of moisture, fat, protein, carbohydrate, ash, acidity and crude fibre respectively.

Nutrients (%)	Cow milk	Safflower milk
Flavour	Mild sweetish	Bland
Colour	Pale yellow	Greyish white
Moisture	87.59	89.90
Fat	3.63	5.48
Protein	3.50	1.77
Carbohydrate	4.12	2.36
Acidity (% LA)	0.12	0.03
Ash	0.65	0.45
Crude fibre	Absent	0.04

Table 1: Physicochemical characteristics of cow milk and safflower milk.

The safflower milk had lower content of ash and protein as compared to cow milk, while it had higher content of fat. The values obtained were on par with the results of [8]. The bland taste of safflower milk might be due to the absence of lactose, a sweet tasting oligosaccharide and smaller amounts of polysaccharides provides mild sweetness to the cow milk. Safflower seeds are reported to contain lower percentage of carbohydrates than the staple cereals [9].

Effect of Various Levels of Safflower Milk on the Sensory Attributes of Dahi

The sensory score for colour and appearance of control was 8.40 as against 8.23, 8.00 and 4.97 for experimental samples blended with safflower milk and cow milk at 20:80, 30:70 and 40:60 ratios respectively (Table 2). Increase in the level of safflower milk in blend resulted in decrease in colour and appearance scores. The reduction in appearance score probably due to the greyish white colour of the safflower milk and its high moisture content. Similar findings with respect to decrease in the sensory score were reported by [10] in the preparation of flavoured milk from cow milk and safflower milk blend.

Levels of SM:CM	Colour and appearance	Body and texture	Flavour	Sourness	Overall acceptability
Control	8.40 ^a	8.79 ^a	8.85 ^a	8.63 ^a	8.51 ^a
20:80	8.23 ^a	8.05 ^a	7.80 ^a	8.05 ^b	8.40 ^a
30:70	8.00 ^a	8.00 ^a	7.75 ^a	7.68 ^c	8.48 ^a
40:60	4.97 ^b	3.95 ^b	3.95 ^b	2.96 ^d	3.95 ^b
CD (P ≤ 0.05)	0.97	1.07	1.07	0.37	0.92

Table 2: Effect of various levels of safflower milk on the sensory attributes of dahi.
*Similar superscripts indicate no significant difference

Dahi sample incorporated with 20 and 30 percent safflower milk showed firm curd as well as good body and texture which may be due to the globular protein that help in the gel formation. The reduction in body and texture score above 30 percent level could be attributed to the low concentration of total solids and absence of casein and whey protein in safflower milk. Reduction in score may also be due to increase in whey separation with increase in safflower milk level which decreases consistency of the product.

Decrease in flavour score at higher level of safflower milk blending could be attributed to the bland, slight oily flavour caused by safflower milk. The value obtained in the present investigation are comparable with values reported by [10] and also comparable with [11], in the flavoured milk prepared from cow milk blended with safflower milk and kalakand prepared from buffalo milk blended with safflower milk respectively. The mean sensory scores for sourness of the dahi were lowered from 8.05 to 2.96 with increase in safflower milk level from 20 to 40 percent which could be due to dilution effect of safflower milk and lesser lactose available for the acid production that might have caused lesser sourness in dahi.

A drastic decrease in the overall acceptability score was found in case of 40 percent safflower milk blended dahi. At higher level of safflower milk blending, product had lesser consistency with whey separation, as a result product scored lower sensory score of 3.95 out of 9.0 for overall acceptability. The values obtained in the present investigation are comparable with values reported by [12], in the case of preparation of kheer from safflower milk blended with buffalo milk. Maximum overall acceptability score was obtained for dahi with 30 percent safflower milk blended with 70 percent cow milk as compared to all safflower milk-cow milk blended dahi. There was no significant difference (P ≥ 0.05) in the values of sensory attributes of dahi except in case of 40:60 blended dahi.

Effect of Various Levels of Safflower Milk on Physicochemical Characteristics of Safflower Milk Blended Dahi

Increase in safflower milk level in blend caused increase in fat content (Table 3). It was observed that fat content in cow milk was lower than that of safflower milk. Safflower milk being a rich source of fat, contributed to fat content of the experimental dahi samples. The protein content followed a decreasing trend with increased level of safflower milk, as the safflower milk had low protein content (1.77%).

Levels of SM:CM	Fat (%)	Protein (%)	pH	Titrateable acidity (% LA)	Total solid (%)	Syneresis (%)
Control	3.57 ^a	3.34 ^a	4.45 ^a	1.0 ^a	12.55 ^a	5.13 ^a
20:80	3.44 ^a	3.15 ^a	4.53 ^a	0.97 ^a	12.52 ^b	5.24 ^a
30:70	3.59 ^a	2.98 ^a	4.60 ^a	0.85 ^a	11.83 ^c	5.38 ^a

40:60	3.76 ^a	2.81 ^a	5.00 ^a	0.60 ^b	11.10 ^d	8.72 ^b
CD (P≤0.05)	1.02	0.75	1.26	0.19	0.56	0.32

Table 3: Effect of various levels of safflower milk on physicochemical characteristics of safflower milk-cow milk blended dahi.

*Similar superscripts indicate no significant difference

The control dahi sample showed a lower pH of 4.45 as compared to the dahi prepared by 40 per cent blending of safflower milk with cow milk (5.00). pH is an important parameter which influences the viability of starter bacteria in all the cultured dairy products. However, decrease in the acidity was observed with increase in the safflower milk proportion. This decreasing trend in the acidity might be due to the absence of lactose in the safflower milk, which is necessary for the fermentation. The total solid content of the control was higher than the experimental samples *viz.*, 12.55, whereas the total solid content for experimental samples ranged from 12.52 to 11.10 percent. There was a gradual decrease in the total solid contents, which may be due to the low total solid content of safflower milk as compared to cow milk. Syneresis or wheying off is a common phenomenon in the fermented milk products like yoghurt and dahi. There was an increase in syneresis in the safflower milk blended dahi samples with increase in the safflower milk level due to the lowered level of milk proteins. Statistically, there was positive effect of safflower milk addition on the rate of syneresis. [13] reported similar results in the preparation of shrikhand from safflower milk blended with buffalo milk.

Effect of Levels of Lactic Culture on the Sensory Attributes of Safflower Milk Blended Dahi

Colour and appearance was good for product inoculated with 1.5 percent level of lactic culture (Table 4). Increased culture addition caused increased acidity and wheying off that reduced score for colour and appearance. The dahi inoculated with 2 percent lactic culture showed lack of uniform appearance due to wheying off. This defect could be the reason for reduction in sensory score of colour and appearance. Dahi prepared by inoculating 1.5% level of lactic culture was found to have better body and texture with maximum body and texture score. This was due to the fact that, dahi was practically free of whey separation, body was firm and texture showed uniformity. [14], ascribed it to the increased lactic culture level that resulted in loose body due to presence of more whey pockets.

Levels of lactic cultures* (%)	Colour and appearance	Body and texture	Flavour	Sourness	Overall acceptability
Control	8.72 ^a	8.17 ^a	8.48 ^a	8.69 ^a	8.69 ^a
1.0	8.53 ^a	8.33 ^a	8.00 ^{ab}	8.25 ^{ab}	8.32 ^{ab}
1.5	8.88 ^a	8.50 ^a	8.05 ^{ab}	8.78 ^a	8.75 ^a
2.0	8.37 ^a	8.10 ^a	7.17 ^b	7.33 ^b	7.50 ^b
CD (P ≤ 0.05)	1.95	1.47	1.04	1.05	1.18

Table 4: Effect of various levels of lactic culture on the sensory attributes of safflower milk-cow milk blended dahi

*Similar superscripts indicate no significant difference.

Dahi inoculated with 2 percent lactic culture showed decrease in the flavour score of safflower milk blended dahi. Increase in the level of incorporation of lactic culture resulted in increased the titratable acidity. This may be the reason for the reduction of flavour and sourness score with increase in the level of lactic culture addition. Dahi prepared by inoculating 2 percent level of lactic culture showed minimum score compared to the control, 1 and 1.5 percent lactic culture inoculated dahi samples. The results found in present investigation are corroborative with the results of [15], in the dahi incorporated with foxtail millet flour.

The maximum overall acceptability score (8.75) was awarded to the sample inoculated with 1.5 percent level of lactic culture, which had comparatively higher score for colour and appearance, body and texture, flavour and sourness compared to the control and other experimental samples. Dahi inoculated with 1 percent lactic culture showed lesser score which may be ascribed to lesser acid production whereas 2 percent lactic culture inoculated sample also showed lesser overall acceptability which may be due to higher acid production.

Effect of Various Levels of Probiotic Cultures on Sensory Attributes of Safflower Milk Blended Dahi

The scores for colour and appearance were good for products inoculated with 3, 5% level of *Lactobacillus acidophilus* LA-5 and 5% level of *Bifidobacterium bifidum* BB-12. Whereas 5 percent *Bifidobacterium bifidum* inoculated dahi was higher i.e. 8.50 as compared to the control and experimental samples (Table 5). This may be due to the fact that, dahi was practically free of whey separation with a firm body and homogeneous texture. Best results for flavour, body and texture were obtained for the sample incubated with 5 percent *Bifidobacterium bifidum* probiotic culture. Increase in the level of incorporation of *Bifidobacterium bifidum* culture re-

sulted in increased production of acetic acid and lactic acid. This may be the reason for increase in flavour score for higher *Bifidobacterium bifidum* culture addition. The sensory score for sourness of 5 percent *Bifidobacterium bifidum* inoculated dahi was higher i.e. 8.50.

Inoculation of *Lactobacillus acidophilus* at 3 and 5 percent showed very high developed acidity that may be the reason for reduced sensory score for sourness attribute. The sample inoculated with 3 percent *Bifidobacterium bifidum* and 3 percent mixed culture failed to meet desirable acidity level whereas 5 percent mixed culture showed higher acidity as compared to 3 and 5 percent of *Lactobacillus acidophilus*. The maximum overall acceptability score of 8.33 was awarded to the sample inoculated with 5 per cent level of *Bifidobacterium bifidum*, which had comparatively higher score for colour and appearance, body and texture, flavour and sourness compared to the control and experimental samples. No significant ($P \leq 0.05\%$) difference was observed in the overall acceptability scores of dahi prepared using different levels of probiotics.

Levels of probiotic cultures	Colour and appearance	Body and texture	Flavour	Sourness	Overall accept-ability
Control	8.05 ^a	8.10 ^a	8.40 ^a	8.33 ^a	8.07 ^a
3% LA	8.17 ^a	7.85 ^a	6.82 ^{ab}	6.54 ^a	7.35 ^a
5 % LA	8.03 ^a	8.17 ^a	6.33 ^b	6.37 ^a	7.87 ^a
3% BB	7.82 ^a	6.78 ^a	7.25 ^{ab}	6.75 ^a	7.12 ^a
5%BB	8.33 ^a	8.50 ^a	8.17 ^{ab}	8.50 ^a	8.33 ^a
3 % Mix	6.67 ^a	6.41 ^a	6.80 ^{bc}	6.50 ^a	7.30 ^a
5 % Mix	7.14 ^a	7.14 ^a	6.30 ^{bc}	6.40 ^a	7.70 ^a
CD(P≤0.05)	1.86	2.29	1.62	2.31	1.88

Table 5: Effect of Various Levels of Probiotic Cultures on Sensory Attributes of Safflower Milk- Cow Milk Blended Dahi.

All the values are average of 3 trials

LA: *Lactobacillus acidophilus* LA-5

BB: *Bifidobacterium bifidum* BB-12

Mix: *Lactobacillus acidophilus* LA-5 + *Bifidobacterium bifidum* BB-12 in equal proportion

Incubation period: 30°C/8 h.

*Similar superscripts indicate no significant difference

Physicochemical Characteristics of Optimized Probiotic Dahi

The optimized safflower milk blended probiotic dahi was found to contain 12.45, 3.73, 8.72, 3.12, 2.24 and 0.60 percent of total solid, fat, solid not fat, protein, lactose and ash respectively (Table 6). The pH of optimized dahi was 4.6 and titratable acidity 0.82% lactic acid. [16] reported that incorporation of safflower milk did not show much effect on the SNF content of the optimized dahi. The control dahi showed 12.41, 3.63, 8.78, 3.50, 2.12 and 0.65 percent of total solid, fat, solid not fat, protein, lactose and ash respectively. The pH of optimized dahi was 4.5 and titratable acidity 0.80 % lactic acid.

Constituents	Safflower milk-cow milk blended dahi (30:70) %	Control dahi %
Total solids	12.45	12.41
Fat	3.73	3.63
SNF	8.72	8.78
Carbohydrate	2.24	2.12
Protein	3.12	3.50
Titratable acidity (% Lactic Acid)	0.82	0.80
Ash	0.60	0.65
pH	4.6	4.5

Table 6: Physicochemical characteristics of the optimized probiotic dahi

All the values are average of 3 trials

Conclusion

From the present study, it may be concluded that a probiotic dahi containing 5 percent *Bifidobacterium bifidum* with superior nutritional values may be prepared using blends of safflower milk and cow milk at 30:70 proportion which is in par with control dahi with respect to physicochemical and sensory characteristics. It will be nutritionally superior besides being cost effective [17,18].

Conflict of Interest

“Authors wish to confirm that there are no known conflicts of interest associated with this publication”.

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