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OPTIMIZATION OF CARROT ENRICHED PROBIOTIC SHRIKHAND

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Abstract: Fermented milk products are most popular in the world in view of their high nutritional and therapeutic value. Fermentation is also a means of preserving nutrients in milk and various fermentation products are developed. Shrikhand is also an indigenous fermented milk product originated from Maharastra. This research study contributes to optimization of carrot enriched shrikhand using wild strains of probiotic and non-probiotic cultures with shelf study at room and refrigeration temperature. The lactic bacterial isolates obtained from dahi samples, *Lactococcus lactis* ssp. *lactis* LC1; *Streptococcus thermophilus* ST1; *Leuconostoc mesenteroides* ssp. *mesenteroides* LEU1 and *Lactobacillus fermentum* LB4 showed in-vitro acid and bile resistance. The probiotic group included *Lactococcus lactis* ssp. *lactis* LC1, *Streptococcus thermophillus* ST1, *Leuconostoc mesenteroides* ssp. *mesenteroides* LEU1 and *Lactobacillus fermentum* LB4 while non-probiotic group comprised of *L. lactis* ssp. *lactis* LC3, *L. lactis* ssp. *lactis* bv. *diacetylactis* LC5, *S. thermophillus* ST2 and *L. fermentum* LB2. Probiotic shrikhand prepared using heat treated whole milk (control), whole milk with carrot juice (7.5:1) and whole milk with carrot pomace (9:1) showed 27.8%, 29.3%; 30.0% yield of chakka respectively. Carrot juice enriched probiotic shrikhand scored more with overall acceptability followed by carrot pomace enriched shrikhand and control.

Keywords: Fermented milk products, Lactic Acid Bacteria, Climatic condition, Isolates, optimized probiotic shrikhand.

Introduction

Shrikhand is one of the indigenous fermented milk product of India. Shrikhand is a semisolid, sweetish sour, wholesome indigenous fermented milk product popular in the states of Gujarat, Maharastra, Karnataka and some parts of north India. Shrikhand, obtained from dahi, contains most of the valuable constituents of milk such as protein, fat, minerals, vitamins and an appreciable amount of B-complex vitamins, particularly riboflavin and folic acid.

Carrot is a variable biennial root crop containing nutrients like carbohydrates, proteins and dietary fiber. Carrot has got health benefits such as stimulation of probiotics due to prebiotic components such as panthothine acid, oligosaccharide and arabinogalactans. Carrot contains some free sugars like sucrose, glucose, xylose and fructose. The bright orange colour of carrot is due to the presence of β-carotene of 8285 µg. Carrot does not contain starch and the taste is due to glutamic acid. Carrot contains some traces like succinic acid, α-ketoglutaric

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acid, lactic acid and glycolic acid. The major phenolic acid is caffeic acid. Carrot is rich in potassium accounting for 320 mg and helps in diuretic property and others like sodium, phosphorous, magnesium are also present. The pectin content of carrot, which contains acid oligosaccharide, a prebiotic is around 0.8g (Gopalan *et al.*, 1989). Health benefits of carrot includes antioxidant property; anti-atherosclerotic property; anti-aggregatory property; anti-inflammatory property and diuretic property (Immerzeel, 2004). An attempt has been made in order to enrich the probiotic shrikhand with the carrot which act as prebiotic nature to the product.

MATERIALS AND METHODS

The shrikhand was prepared using the cultures isolated from the dahi sample. The whole milk was procured from the Student Experimental Dairy Plant (SEDP), Dairy Science college, KVAFSU, Hebbal, Bengaluru and the carrots (*Daucus carrota*) of nantes variety were brought from the local market (Hopcom's). The carrots were washed and removed the outer skin using clean knife then cut into small pieces and steam cooked, cooled and put into the grinder, filtered using clean dry muslin cloth to obtain the juice and pomace.

One set of whole milk having 3.2% Milk fat and 7.65% SNF was mixed with the carrot juice having Total Solids (TS) of 91% (IS: 9532: 1980) and pectin of 0.3% while to another set of whole milk was mixed with carrot pomace having TS of 93.7% (IS: SP: Part II, 1981) and pectin of 1.2% and finally TS of the enriched juice and pomace shrikhand was found to be 60.0% each. The whole milk was mixed with carrot juice at the ratio of 2.5:1; 5:1; 7.5:1 and 10:1 and whole milk was mixed carrot pomace at the ratio of 3:1; 6:1; 9:1 and 12:1.

Then the enriched milk was heated to 85°C/30 min, subsequently cooled to 30°C and inoculated with combination of probiotic cultures such as *L.lactis* ssp. *lactis* LC1 +*S.thermophillus* ST1+ *Leuconostoc mesenteroides* ssp. *mesenteroides* LEU1 **+** *L. fermentum* LB4 at 0.25 per cent each and combination of non-probiotic cultures such as *L.lactis ssp. lactis* LC3+*L. lactis* ssp. *lactis* bv. *diacetyl lactis* LC5+ *S.thermophillus* ST2 + *L. fermentum* LB2 at 0.25 per cent each separately.

The set dahi transferred into a sterile muslin cloth and hung for 4 hrs at room temperature in a laminar air flow unit. The semi-solid mass (chakka) obtained was transferred to a sterile container and used in the shrikhand preparation.

A known quantity of chakka was taken onto a sterile plate. For every 100g of chakka 40g of powdered sugar was added and mixed well with chakka using sterile spoon. The kneading was continued until no lumps were observed in the product. The products thus obtained were

packed in Polysterine cups (100ml), transferred to refrigerator and cold served for organoleptic analysis by a panel of judges.

Salwa *et al.,* (2004), suggested that yield and quality of carrot juice that contains high amount of α and β-carotene vary with the pre-treatment like blanching solution and blanching times (1–5 min). Excellent quality carrot-yoghurt could be prepared by blending milk in different proportions having 5–20% carrot juice before fermentation (Sharma *et al.,* 2009).

Sensory evaluation of shrikhand samples

The product was given for organoleptic analysis in order to select the product of good quality. The parameters used in the score card were colour and appearance, body and texture, flavour and overall acceptability.

Fat: Fat content in whole milk was analyzed by IS: 1224(1958)

Titratable Acidity: Titratable acidity of dahi, chakka and shrikhand were carried out as per the standard procedure given in IS: 1479, part 1 (1960). Titratable acidity of curd was determined as per the standard procedure given in IS: 7035 (1973). Titratable acidity of chakka and shrikhand was determined as per the ISI procedure (IS: 1479 - I and II).

Total solids: Total solids of whole milk and shrikhand samples were estimated by gravimetric method by IS: SP: 18(part XI)- 1981.

Direct Microscopic Count (DMC- Harrigan, 1998)

Samples to be analyzed were mixed well and diluted to 1:10 by using normal saline. Sample of 0.01ml (10 µl) was transferred using a Breed's pipette onto a marked slide and smear was prepared by spreading evenly in the marked area of 1sq.cm. The smear was fixed using ethanol for 2min., treated with xylene for 2 min to remove cream layer and finally stained by using borax methylene blue for 5 min, then observed under the oil immersion objective and organisms were counted in each of the field. The average number of organisms per field was calculated and multiplied by microscopic factor determined by using stage micrometer and dilution factor and expressed as number of organisms per gram of the sample.

RESULTS AND DISCUSSION

In order to optimize carrot enriched shrikhand preparation, the probiotic culture containing *Lactococcus lactis* ssp. *lactis* LC1, *Streptococcus thermophilus S*T1, *Leuconsotoc mesenteroides* ssp. *mesenteroides* LEU1 and *Lactobacillus fermentum* LB4 were inoculated to heat treated whole milk, whole milkwith carrot juice (2.5:1; 5:1; 7.5:1& 10:1) and whole milk with carrot pomace (3:1; 6:1; 9:1& 12:1) at 0.25% each, accounting to 1% and incubated at 30^0C.

The whole milk was set at 10 hours while whole milk(WM) with carrot juice(CJ) of 7.5:1 and whole milk with carrot pomace(CP) of 9:1 took 8 hours to set the milk with TA of 0.80(WM), 0.96% (WM:CJ) and 1.02% (WM:CP) respectively. Direct Microscopic Count (DMC) for the same combinations were of 7.98 (WM), 8.20 (WM:CJ) and 8.60 log_{10}/g (WM:CP) respectively. Other combinations took 10 hours to set the milk. Selection of the carrot enrichment proportion was based on higher DMC of the associative cultures both in case of probiotic and non-probiotic cultures. Yield of chakka was 27.8 for whole milk followed by 29.3 and 30.0 for whole milk with carrot juice (7.5:1) and whole milk with carrot pomace (9:1) respectively (Table 1).

The whole milk as control, milk and carrot juice at the ratio of 7.5 to 1; milk and carrot pomace of 9:1 were selected and used for making shrikhand with both probiotic and non-probiotic with 0.25% inoculum. Probiotic and Non-probiotic cultures set the whole milk at 10 hours and 8 hours for milk enriched with carrot extract. The acidity of control shrikhand was 0.82 and 0.72% lactic acid and DMC of 7.90 and 7.60 log_{10}/g for probiotic and non-probiotic cultures respectively (Table. 2. A).

The carrot juice enriched shrikhand made using probiotic and non-probiotic cultures showed acidity of 0.96 and 0.81 with DMC of 8.20 and 7.88 log_{10}/g respectively for probiotic and non-probiotic cultures (Table. 2. B). The carrot pomace enriched shrikhand made using probiotic and non-probiotic cultures showed acidity of 1.02 and 0.84 %LA, with DMC of 8.60 and 7.99 log_{10}/g for probiotic and nonprobiotic cultures respectively which was higher compared to the other ratios of carrot juice and carrot pomace in whole milk (Table. 2. C).

Whole milk with carrot juice (7.5:1) and carrot pomace (9:1) that gave good yield of chakka also helped in the early setting of dahi as well as showed good DMC values with acceptable titratable acidity. Significant difference occurred among the ratios of carrot extract with respect to the yield of chakka. Hence the same ratios of carrot extract in whole milk were adopted for shrikhand preparation using non probiotic culture (Table 2).

Milk enriched with carrot juice 7.5:1 inoculated with probiotic culture showed more acidity with higher DMC (8.20 log_{10}/g) compared to carrot juice enriched shrikhand made using non probiotic culture. The same trend was noticed in milk enriched with carrot pomace inoculated with probiotic culture and shrikhand was prepared out of it. Carrot pomace enriched shrikhand at the ratio of 9:1 showed more acidity of 1.02% when probiotic culture was used compared to non-probiotic (0.84%) along with higher DMC (8.60 log_{10}cfu/ml).

Srinivasa., (2008), could able to get an yield of 27.8% after enriching the milk with 5% whey protein concentrate , 5% honey and 15% pineapple pulp. Srinivasa (2008) found viable count of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* as 7.04 and 7.62 cfu/g in enriched shrikhand with 5% whey protein concentrate, 5% honey and 15% whey protein concentrate. Excellent quality carrot-yoghurt could be prepared by blending milk in different proportions having 5–20% carrot juice before fermentation (Sharma *et al.,* 2009).

Organoleptic evaluation of carrot enriched shrikhand:

The samples of whole milk shrikhand, carrot enriched shrikhand (carrot juice and carrot pomace enriched) made using probiotic and non-probiotic cultures were given to a panel of judges for judging the sample. The parameters used in the judging were colour and appearance, body and texture, flavour and overall acceptability using 9-point hedonic scale. The colour and appearance of milk, carrot juice and carrot pomace enriched probiotic shrikhand gave the scores as 8.00, 8.05 and 8.10 with body & texture of 7.97, 8.10, 8.05 and with overall acceptability of 8.00, 8.20 and 8.00 respectively. The scores of Non-probiotic shrikhand for whole milk, carrot juice and carrot pomace enriched was found to be 7.95, 8.00 and 7.80 for colour and appearance followed by 7.93, 7.95, 7.97 with overall acceptability of 7.95, 8.10, 8.00 respectively (Table 3).

Carrot juice enriched probiotic shrikhand showed good organoleptic quality followed by carrot pomace enriched and control shrikhand. The carrot enriched shrikhand prepared using probiotic and non-probiotic cultures showed similar organoleptic quality with respect to whole milk shrikhand as the values obtained for colour, Body and texture, flavour and Overall acceptability had no significant difference statistically. But visual variation in the colour was noticed by the panel of judges in carrot enriched shrikhand compared to control sample irrespective of cultures used in the preparation. The shrikhand enriched with carrot juice (8.20) was more acceptable with respect to overall acceptability than carrot pomace enriched shrikhand (8.10) and control shrikhand (8.00) prepared using probiotic culture than non-probiotic culture. A comparable study by Srinivasa (2008) showed that enriched shrikhand with 5% whey protein concentrate, 5% honey and 15% pineapple pulp gave significantly maximum overall acceptability when compared to control.

Conclusion

Fermentation of milk product is the one of the best method of preserving nutrients and exerts added good therapeutic value when added with the probiotic cultures. Probiotic shrikhand prepared using heat treated whole milk (control), whole milk with carrot juice (7.5:1) and

whole milk with carrot pomace (9:1) showed 27.8%, 29.3%; 30.0% yield of chakka respectively. The carrot juice enriched probiotic shrikhand was very good followed by the carrot pomace enriched shrikhand. The product prepared was good with the sensory aspects wherein carrot juice scored more (8.05) followed by carrot pomace (8.00) and whole milk probiotic shrikhand (8.00).

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Table 1: Optimization of the carrot enriched probiotic shrikhand

A) Optimization of shrikhand using carrot juice

Milk : juice	Setting time of milk (h)	Yield of chakka (%)	Titratable acidity of shrikhand (% LA)	DMC (log_{10}/g)	C.D for chakka yield
Only milk	10	27.80	0.80	7.98	2.73
2.5:1	10	30.20	0.85	8.06	
5.0:1	10	29.80	0.90	8.12	
7.5:1	**8**	**29.30**	**0.96**	**8.20**	
10: 1	8	**28.00**	0.93	8.10	

B) Optimization of shrikhand using carrot pomace

Milk : Pomace	Setting time of milk (h)	Yield of chakka (%)	Titratable acidity of shrikhand (% LA)	DMC (log_{10}/g)	C.D for chakka yield
Only milk	10	27.80	0.80	7.98	3.16
3:1	10	29.00	0.86	8.11	
6:1	10	30.50	0.96	8.19	
9:1	**08**	**30.00**	**1.02**	**8.60**	
12:1	08	28.30	0.90	8.10	

- All the values are average of 3 trials.

Note: * Probiotic culture used for dahi preparation are *Lactococcus lactis* ssp. *lactis* LC1 + *Streptococcus thermophillus* ST1 + *Leuconostoc mesenteroides* ssp. *mesenteroides* LEU1 and *L.fermentum* LB4 each inoculated at 0.25% each.

Table 2: Optimized carrot enriched shrikhand using probiotic and non-probiotic cultures

A. Shrikhand using whole milk

Milk	Combination of lactic cultures	% inoculum	Setting time of milk (h)	% LA of shrikhand	DMC log_{10}/g
Whole milk	**Probiotic cultures** *Lactococcus lactis ssp. lactis* LC1 + *Streptococcus thermophillus* ST1 +*Leuconostoc mesenteroides ssp*. mesenteroides LEU1 + *Lactobacillus fermentum* LB4	0.25 each	10	0.82	7.90
	Non-probiotic cultures *L.lactis ssp. lactis* LC3 + *L.lactis* ssp. *lactis* bv. *diacetylactis* LC5 + *S.thermophillus* ST2 + *L.fermentum* LB2	0.25 each	10	0.72	7.60

B. Shrikhand using carrot juice

Milk: Juice	Combination of lactic cultures	% inoculum	Setting time of milk (h)	% LA of shrikhand	DMC log_{10}/g
7.5:1	**Probiotic cultures** *Lactococcus lactis ssp. lactis* LC1 + *Streptococcus thermophillus* ST1 + *Leuconostoc mesenteroides ssp.* mesenteroides LEU1 **+** *Lactobacillus fermentum* LB4	0.25 each	8	0.96	8.20
	Non-probiotic cultures *L.lactis ssp. lactis* LC3 + *L.lactis* ssp. *Lactis* bv. *diacetylactis* LC5 + *S.thermophillus* ST2 + *L. fermentum* LB2	0.25 each	8	0.81	7.88

C. Shrikhand using carrot pomace

Milk: Pomace	Combination of cultures	% Inoculum	Setting time of milk (h)	% LA of shrikhand	DMC log_{10}/g
9:1	**Probiotic cultures** *Lactococcus lactis ssp. lactis* LC1 + *Streptococcus thermophillus* ST1 + *Leuconostoc mesenteroides ssp. mesenteroides* LEU1**+** *Lactobacillus fermentum* LB4	0.25 each	8	1.02	8.60
	Non-probiotic cultures *L. lactis ssp. lactis* LC3 + *L. lactis* ssp. *lactis* bv. *diacetylactis* LC5 + *S. thermophillus* ST2 + *L. fermentum* LB2	0.25 each	8	0.84	7.99

Note: All the values are average of 3 trials.

Table 3: Organoleptic evaluation of control and carrot enriched shrikhand

Type of milk used	Colour and appearance		Body and texture		Flavour		Overall Acceptability	
	PC	NPC	PC	NPC	PC	NPC	PC	NPC
Only Milk	8.00	7.95	7.97	7.93	7.85	7.65	8.00	7.95
7.5(Milk):1 (carrot juice)	8.05	8.00	8.10	7.95	7.90	7.75	8.20	8.10
9(Milk):1 (carrot Pomace)	8.00	7.80	8.05	7.97	7.80	7.70	8.10	8.00
C.D	0.65							

- All the values are average of 3 trials.

Note: PC - Probiotic cultures: *Lactococcus lactis ssp. lactis* LC1 + *Streptococcus thermophillus* ST1 + *Leuconostoc mesenteroides* ssp. *mesenteroides* LEU1 + *Lactobacillus fermentum* LB4

NPC - Non-probiotic cultures: *L.lactis ssp. lactis* LC3 + *L.lactis* ssp. *lactis* bv. *diacetylactis* LC5 + *S. thermophillus* ST2 +*L. fermentum* LB2.