

STUDIES ON SUITABILITY OF INCORPORATING PROBIOTICS IN MANGO-BASED KULFI- A POPULAR INDIAN FROZEN DESSERT

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Abstract

*Kulfi is popular milk based frozen dessert. The viability of probiotic and their stability in various food products containing substrates like milk, fruits, or vegetables as well as in products which are stored at very low temperatures including ice cream and other frozen desserts are key focus areas for research. Therefore, an attempt has made in present investigation to evaluate suitability of incorporation probiotics in mango-based Kulfi (a frozen dairy dessert/candy of Indian subcontinent). The hardness for Kulfi having 10, 15 and 20 per cent mango pulp was significantly higher than control. The probiotic Kulfi had 8.76% fat at 2% inoculum level which decreased in a linear proportion being 8.69% and 8.58% at 3 and 4% inoculation level of probiotic culture, respectively. Statistically, the differences in fat content of probiotic Kulfi due to treatments were significant. The results of current study revealed that in kulfi mix addition of 15% Alphonso mango pulp and 3% mixed probiotic cultures *L. acidophilus* and *L. casei* (50:50 proportion) yielded the product with superior quality, having more viability count of probiotics (8.75 and 8.20 log cfu/g, respectively of *L. acidophilus* and *L. casei*) after storage as compared to other treatment combinations. The developed product was effective in terms of nutritional quality, without compromising on the taste of the kulfi available locally.*

Keywords: kulfi; frozen dairy dessert; probiotics; value addition; cell viability; sensory evaluation

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1. INTRODUCTION

Kulfi, also known as Malai *Kulfi* /Malai-ka-burf is a popular Indian frozen milk product prepared from cow or buffalo milk and/or a combination thereof. It closely resembles ice cream in composition; however it does not contain air (Nalkar, 2012; Singh et al., 2017a; Nizam and Rai, 2018). Traditionally, *Kulfi* is prepared by concentrating sweetened and flavoured milk by slow heating with continuous stirring until its volume is reduced by a half. It comes in various flavours, including rose, mango, cardamom, saffron (*kesar* or saffron), strawberry, and pistachio; as well as supplemented with fruit pulp of mango, apple, orange, strawberry, and peanut.

The awareness and popularity of probiotic is increasing enormously among the global population in last few years. These bacteria are

preferred over antibiotics by being preventive, non-invasive and free of undesirable side effects. Probiotic organisms to be used should be viable and must be minimum 10^6 cells per dose of a serving to exert a beneficial effect (Boylston et al., 2004; Korbekandi et al. 2011). The viability of probiotic and their stability in various food products containing substrates like milk, fruits, or vegetables as well as in products which are stored at very low temperatures (such as ice cream and other frozen desserts) are key focus areas for research (Patel, 2017). There is also a greater need for selection and developing starter culture, particularly mixed culture for developing a novel probiotic dairy product.

Mango (*Mangifera indica* L.) cv. Alphonso is the most popular fruit (king of fruit crops) of tropics due to its high palatability, excellent taste, flavour, nutritional and therapeutic

importance (Shah et al., 2010). It thrives and yields best under hot and humid agro climatic conditions of the India and nearby Asian countries. Alphonso mango contains high β -carotene, a precursor of Vitamin A; further it is a rich source of the vitamin B complex, especially folic acid and vitamin C (Tharanathan et al., 2007). In general the ripe mangoes are reported to have 73.0-86.7% moisture, 0.5-1.0% protein, 0.1-0.8% fat, 11.6-24.3% carbohydrate, 0.412% calcium, 0.195% phosphorous, 50 ppm iron, 6375-20750 IU vitamin A ($\mu\text{g}/100\text{ g } \beta$ -carotene), 50 mg/100 g riboflavin and 0.12-0.38% acidity (USDA, 2011).

Keeping in view the above mentioned therapeutic importance of probiotics and nutritional significance of Alphonso, an investigation was planned aimed at suitability of incorporation of probiotic organisms in mango-based *Kulfi*. The basic objectives of the current research were to optimize the level of Alphonso mango pulp as well as probiotic cultures (in single and mixed form) in *Kulfi* and to standardization of method of manufacture of probiotic *Kulfi*.

2. MATERIALS AND METHODS

Bacterial strain and culture media:

Freeze dried cultures viz., *Lactobacillus acidophilus* (015) and *Lactobacillus casei* (017) were procured from the National Collection of Dairy Cultures, NDRI, Karnal (Haryana). These were sub cultured and maintained in the laboratory of Biotechnology centre of the University at Dapoli. They were maintained and propagated in sterilized reconstituted skim milk (10% Total Solids) followed by incubation at 37°C for 8 h and stored at 5 \pm 1°C during the entire course of the study. Prior to use, three successive transfers were given to the culture in skim milk for its activation. All bacteriological media were purchased from Sigma (Germany) or Hi-Media (Mumbai, India).

Optimization of Alphonso pulp and probiotics level:

To obtain a probiotic *Kulfi* with appropriate level of the mango pulp, different levels of Alphonso pulp viz., 10%, 15%, and 20% were incorporated in the *Kulfi* mix in order to determine the optimum level of mango pulp in the product. This amount was determined based on the observations of chemical composition (fat, total solids, acidity, protein), rheological quality (hardness), melting quality, sensory quality, and microbiological quality. Further, probiotic cultures viz., *L. acidophilus*, *L. casei* and mixed form of these two were evaluated individually at 2%, 3% and 4% levels in order to optimize their level in the final product. On the basis of earlier mentioned parameters their optimum levels in *Kulfi* were determined.

Preparation of *Kulfi*:

The raw milk was heated to reduce the moisture partially from the milk (75% volume) calculated quantities of 15 per cent sugar, skim milk powder to adjust total solids (TS) and 0.5 percent of gelatin were added to milk to make the mix with 10 to 12% of milk fat and 40% of TS. The stabilizer was dissolved into small quantity of hot water, filtered through a clean muslin cloth and added to the mix. It was then subjected to pasteurization by LTLT method. The pasteurized milk was cooled to room temperature and was kept at 0 to -5 °C for six hours for ageing to hasten the consistency and improve the whipping quality. Subsequently, it was added with mango pulp and mixed well before filling in moulds for freezing. The moulds were kept for freezing having about -8 to -10 °C for freezing. It was periodically shaken. After freezing, the moulds were kept in the deep freeze at -18 to -20 °C temperature for 24 hr. for hardening.

Preparation of probiotic mango *Kulfi* mix:

Based on the optimized amount of Alphonso pulp and culture level, finally probiotic mango *Kulfi* was prepared as shown in flow chart (Figure 1). The probiotics were added to the *Kulfi* mix just after cooling of milk and incubated for 5h at 37 °C prior to addition of mango pulp.

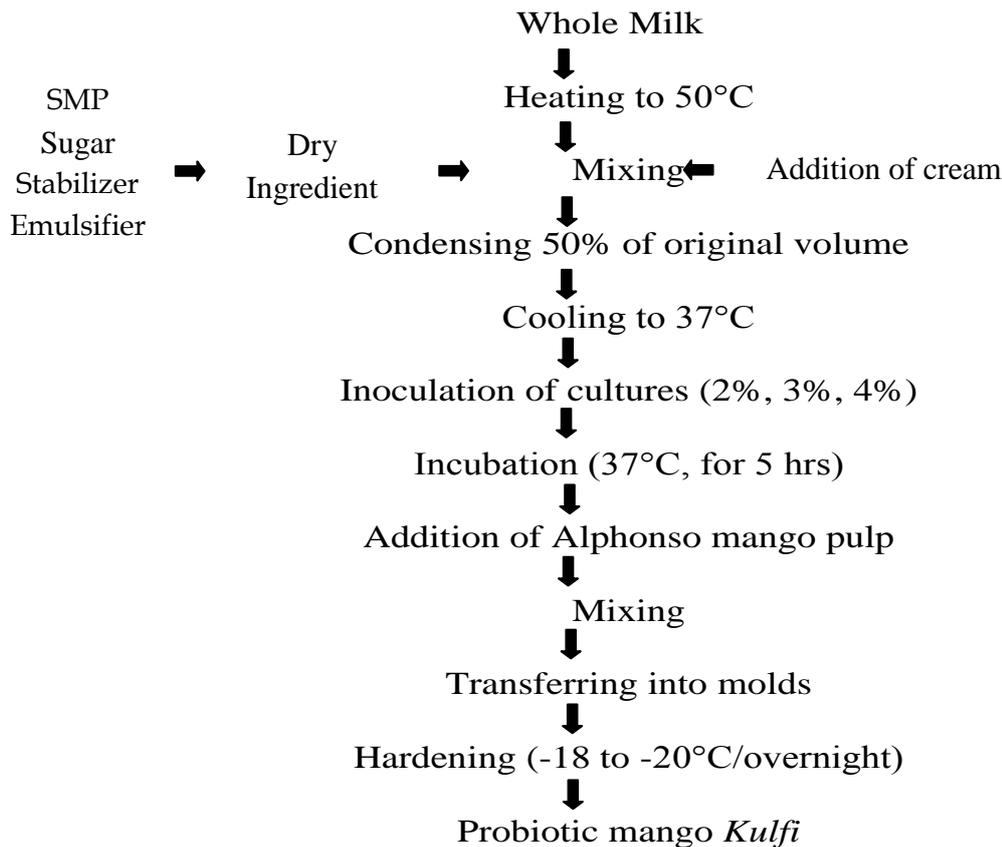


Fig. 1: Flow diagram for preparation of probiotic mango *Kulfi*

Evaluation of physico-chemical properties of probiotic *Kulfi*:

All chemical constituents including acidity, total solids (TS), protein content, and fat content were estimated as per the standard methods (AOAC, 2005). The melting time of *Kulfi* samples was determined by following method of Arbuckle (1986) while the hardness was determined by AIM-514 automatic penetrometer timer (New Delhi, India).

Microbiological analysis of *Kulfi*:

Standard plate counts (SPC), viable *Lactobacilli* count, *E. coli* count, and Psychrotrophic counts were carried out for the finished product. Serial dilutions of the product were prepared and appropriate dilutions were pour-plated in duplicates on plate count agar for both, SPC count and Psychrotrophic count; on de Mann-Rogosa-Sharpe (MRS) agar for lactobacilli count, and on Violet red bile agar (VRBA) for *E. coli* count. All the plates were kept at specific time temperature conditions

and after incubation periods, plates were observed for colony characteristics and numbers of colony forming units (cfu) were enumerated.

Sensory evaluation of *Kulfi*:

All the samples were evaluated for sensory attributes such as color and appearance, body and texture, flavor, and overall acceptability on a nine-point hedonic scale (9 for liking extremely and 1 for disliking extremely) by the panel of discriminative and experienced expert judges was formulated. The products were marked randomly and served in 50 g of sample during each trial.

Statistical analysis of the data:

All the experiments were carried out in three trials (n=3). Data were analyzed for variance by Completely Randomized Design (CRD) as per the methods described by Panse and Sukhatme (1985). The significance was tested at five percent level using 'F' test and CD percent was calculated. The values for

microbial counts were log transformed before analysis.

3. RESULTS AND DISCUSSION

The results of the first phase revealed that amongst different levels of the mango pulp (0, 10, 15 and 20%), incorporation of 15% pulp in *Kulfi* (T_{1c}) yielded the best results with respect to chemical quality, physical properties, sensory qualities and microbial content (Table 1-3). Fat content of the *Kulfi* decreased with the increase of fruit pulp level in the mix, thus control sample (T_{1a}), had the highest (9.32%) fat than remaining three *Kulfi* mixes incorporated with mango pulp. The decrease in protein content of *Kulfi* with increasing levels of fruit pulp may be ascribed to very low protein content of mango where it varied from

0.65 to 0.68 per cent (Chavan, 1997). The average TS content of *Kulfi* in different treatments ranged from 34.20 % to 38.63 % which is very close to that reported by other authors (Salooja and Balchandran, 1982; Singh et al., 2017a). The mean acidity values for *Kulfi* samples under different treatments were 0.27%, 0.29%, 0.30% and 0.31% for T_{1a}, T_{1b}, T_{1c}, and T_{1d}, respectively. These findings are in concurrence with the findings of other authors (Dongale, 2001).

The ability of *Kulfi* samples to resist the melt down is mainly dependent on its freezing point, melting point and the compositional make up. Results revealed that there was gradual decrease in the melting time of *Kulfi* samples with an increase in the levels of mango pulp (Nalkar, 2012).

Table 1: Chemical composition, Melting time and Hardness of mango *Kulfi*

Replication Treatment	Constituent (%)				Melting time (min)	Hardness (mm/ 5 sec)
	Fat	TSS	Acidity	Protein		
T _{1a}	9.32 ^c	38.63	0.27	4.46	35.43 ^c	39.09 ^a
T _{1b}	8.75 ^b	38.20	0.29	4.40	34.14 ^b	42.20 ^c
T _{1c}	8.60 ^{ab}	37.43	0.30	4.37	32.40 ^b	41.72 ^c
T _{1d}	8.45 ^a	37.20	0.31	4.30	28.66 ^a	40.76 ^b
MS.E. ±	0.054	0.730	0.015	0.036	0.240	0.204
C.D. at 1 %	0.218	NS	NS	NS	0.968	0.821
Result					Sig	Sig

Note: Figures having similar superscripts do not differ significantly from each other.

Table 2: Sensory quality of mango *Kulfi* containing different levels of the mango pulp

Treatment	General app. and colour	Rank Total	Flavour	Rank Total	Body and Texture	Rank Total	Overall Accept- ability	Rank Total
T _{1a}	7.31	20	6.56	22	6.88	19	6.92	21
T _{1b}	7.30	18	7.56	20	7.17	17	7.04	21
T _{1c}	7.83	8	8.27	7	8.25	6	8.11	6
T _{1d}	7.61	14	7.67	11	7.34	18	7.53	12
C. D. at 1 %	-	7.625	-	3.968	-	6.511	-	3.302
Results	Sig		Sig		Sig		Sig	

where. T_{1a} – Control (No addition of mango pulp), T_{1b} – Addition of mango pulp at 10% of mix, T_{1c} – Addition of mango pulp at 15% of mix, T_{1d} – Addition of mango pulp at 20% of mix

Table 3: Microbiology quality of mango Kulfi containing different levels of the mango pulp

Replication Treatment	Standard Plate Count (10^5 cfu/g)	<i>E. coli</i> count (10^3 cfu/g)	Psychrotrophic count (10^2 cfu/g)
T _{1a}	2.65 b	1.50 a	0.98 b
T _{1b}	2.07 a	2.00 a	0.48 a
T _{1c}	3.42 c	3.67 b	0.58 a
T _{1d}	3.85 d	4.17 b	0.70 a
MS.E. \pm	0.102	0.404	0.059
C.D. at 1 %	0.414	1.629	0.237
Result	Sig.	Sig.	Sig.

Note: Figures having similar superscripts do not differ significantly from each other

The increase in melting resistance values may be due to the presence of soluble dietary fiber or carbohydrate which forms a complex matrix which binds and holds the water resulting in slow melting (Singh et al., 2017b). The decrease in hardness with increasing level of pulp may be due to decrease in TS and fat content of *Kulfi* with increasing pulp levels. The hardness for *Kulfi* having 10, 15 and 20 per cent mango pulp was significantly higher than control. Similarly, the difference in hardness of 10 and 15 per cent mango pulp *Kulfi* was not significant.

Incorporation of 15% mango pulp in *Kulfi* level had the highest score 7.83, 8.27, 8.25, and 8.11 respectively for general appearance, flavor, body and texture and overall acceptability amongst all the treatments (Nalkar, 2012). The SPC in different treatment varied significantly from each other; the SPC of control sample and treated sample was on par with the BIS standard. There was rise in *E. coli* count and showed positive relationship with fruit pulp levels in the *Kulfi* samples, the values being minimum at 10% (2.00×10^3 cfu/g) and maximum at 20% (4.17×10^3 cfu/g). It is also likely that sugar in mango pulp may be a source of substrate for growth of *E. coli*. The results obtained during this investigation were in agreement with other studies (Arunkumar, 1993; Ashokraju et al., 1989; Giri et al., 2012). The psychrotrophic count in control group was much higher than in *Kulfi* with different levels of mango pulp. The psychrotrophic count might be due to post contamination during preparation. The microbiological results of the *Kulfi* sample were on par with the Bureau of

Indian standards (BIS) and also lower as compared to market samples.

In the second phase, different levels of probiotic cultures were evaluated. The different levels of *L. acidophilus* (0, 2, 3 and 4%) or *L. casei* (0, 2, 3 and 4%) individually or in the form of mixed culture in 50:50 proportion indicated that addition of only *L. acidophilus* (2%), or *L. casei* (4%), or mixed culture (3%) was found optimum to obtain *Kulfi* of desirable quality with regards to chemical composition, sensory parameters, physical properties and microbial status (Table 4-6). The viability of cells after incubation and during storage at -18°C to -20°C for 24 h was retained and was good at these levels of addition.

Based on the results of the first phase and second phase, third phase study was formulated wherein optimum level of the pulp with optimum level of probiotic culture individually or mixed was coupled and evaluated. In this product, probiotic mixed culture and mango pulp were added in the *Kulfi* mix and kept for incubation 5 h at 37°C and then *Kulfi* cones were filled and stored. The viability of probiotics in *Kulfi* mix increased after incubation and also remained viable after storage of *Kulfi* at -18°C to -20°C for 24 h. The results revealed that in *Kulfi* mix addition of 15% the mango pulp and 3% mixed culture of *L. acidophilus* (015) and *L. casei* (017) (50:50 proportion) yielded the product with superior quality, having more viable counts of *L. acidophilus* and *L. casei* after storage as compared to other treatment combinations. In *Kulfi* mix with 15% mango pulp, the viable counts of the probiotic culture (*L. acidophilus* at 2% level) was 8.28 log cfu/g (T_{1c} x T_{2b})

while in another *Kulfi* mix, having probiotic culture *L. casei* (at 4% inoculation level) the viable counts was 8.32 log cfu/g (T_{1c} x T_{3d}). The probiotic culture (mixed culture) at 3 %inoculation level and 15% mango pulp in *Kulfi* mix showed viable counts of *L.*

acidophilus as 9.45 log cfu/g and of *L. casei* as 8.40 log cfu/g (T_{1c} x T_{4c}).

It can be observed that, the control (T_{1c}) mango *Kulfi* (non-probiotic) sample recorded significantly lower melt down time (32.22 min) than those of treated samples (Table 4).

Table 4: Effect of optimum level of mango pulp with optimum level probiotic cultures on chemical composition, Melting time and Hardness of probiotic mango *Kulfi*

Treatment	Chemical constituents (%)				Melting time (min)	Hardness (mm/5sec)
	Fat	Protein				
T _{1c} (control)	9.32	37.44	0.32 a	4.37	32.22 a	41.70 c
T _{1c} x T _{2b}	8.62 a	37.20	0.34 a	4.45	34.83 b	38.78 a
T _{1c} x T _{3d}	8.61 a	37.11	0.41 c	4.39	39.24 d	40.01 b
T _{1c} x T _{4c}	8.60 a	37.17	0.38 b	4.40	37.02 c	39.00 a
MS.E. [±]	0.022	0.324	0.006	0.021	0.166	0.126
C. D. at 1 %	0.866	-	-	0.009	0.652	0.492
Result	Sig	NS	NS	Sig	Sig	Sig

Note- Figures having similar superscripts do not differ significantly from each other.

Table 5: Effect of optimum level of mango pulp and optimum level probiotic cultures on sensory quality of probiotic mango *Kulfi*

Treatment	General app. and colour	Rank Total	Flavour	Rank Total	Body and Texture	Rank Total	Overall Accept-ability	Rank Total
T _{1c}	7.89	32	8.27	27	8.25	26	8.13	30
T _{1c} x T _{2b}	8.15	22	8.39	25	8.32	25	8.25	24
T _{1c} x T _{3d}	8.32	12	8.49	17	8.51	13	8.47	12
T _{1c} x T _{4c}	8.28	14	7.51	11	8.55	10	8.43	14
C. D. at 1 %	-	5.581	-	8.215	-	8.268	-	6.708
Result		Sig		Sig		NS		Sig

Table 6: Effect of optimum level of mango pulp and optimum level probiotic cultures (*L. acidophilus*, *L. casei*, and mixed culture) before and after incubation

Treatment	Viable counts (log cfu/g)							
	Before Incubation		After Incubation		Mixed culture Before Incubation		Mixed culture After Incubation	
	<i>Lb. a</i>	<i>Lb. a</i>	<i>Lb. c</i>	<i>Lb. c</i>	<i>Lb. a</i>	<i>Lb. a</i>	<i>Lb. c</i>	<i>Lb. c</i>
T _{1c}	-	-	-	-	-	-	-	-
T _{1c} x T _{2b}	7.62	8.25	-	-	-	-	-	-
T _{1c} x T _{3d}	-	-	7.88	8.31	-	-	-	-
T _{1c} x T _{4c}	-	-	-	-	7.65	8.75	7.45	8.20

Average melting time in the treated *Kulfi* samples was 34.83 (T_{1c} x T_{2b}), 39.24 (T_{1c} x T_{3d}) and 37.02 (T_{1c} x T_{4c}), minutes at 2, 4, and 3 per cent inoculation levels of probiotic culture with 15% mango pulp, respectively. Shivaprakash (2002) reported that, extent of inoculums was found to have significant effect on hardness of ice cream. With the increase in the levels of inoculums, slight decrease in hardness of ice cream was observed as evident from higher penetration value. Similar observation was made by other researchers (Salem et al., 2005, Giri et al., 2012). It is evident from the Table 5 that *Kulfi* sample of T_{1c} x T_{3d} treatment was superior to rest of the *Kulfi* samples for general appearance, flavor, body and texture and overall acceptability attributes, viz. 8.32, 8.49, 8.51 and 8.47, respectively.

The fat content of the probiotic *Kulfi* decreased with the increase in the level of inoculation of mixed culture in the mix. The probiotic *Kulfi* had 8.76% fat at 2% inoculums level which decreased in a linear proportion being 8.69% and 8.58% at 3 and 4% inoculation level of probiotic culture, respectively. Statistically, the differences in fat content of probiotic *Kulfi* due to treatments were significant.

There was rising trend in the acidity development with an increase in the level of inoculation of cultures and the incubation period up to 5 hr at 37°C. It is revealed that the mean acidity content in *Kulfi* of different treatments was 0.26 (T_{4a}), 0.36 (T_{4b}), 0.42 (T_{4c}) and 0.51% (T_{4d}) at 0 (control), 2, 3, and 4% inoculation levels of mixed culture, respectively. The acidity of probiotic *Kulfi* was higher than control. Further, all the *Kulfi* samples of different treatments differed significantly from each other. Arunkumar (1993) reported that the pre-incubation resulted in an appreciable increase in viable counts of *L. acidophilus*. This increase in the viable count may be due to associative growth and multiplication of cells present in the *Kulfi* mixes. Longer hours of incubation resulted in an increase in acid production and decrease in pH values, causing the product to become slightly sour (Martin and Laroia 1991; Giri et

al., 2012; Nizam and Rai 2018). The penetration value for *Kulfi* of T_{4d} with 4% inoculation was significantly higher than rest of the treatments. The *Kulfi* of T_{4a}, T_{4b} and T_{4c} had statistically similar hardness value.

There was a significant effect on the viability of the *L. acidophilus* cells due to the treatments and it was slightly increased as the inoculation level increased. However, there was no difference in viable count of *L. casei* during storage due to treatments (Nalkar, 2012). The viable count of *L. acidophilus* during storage in 0, 2, 3 and 4% inoculation was observed as 0.00, 9.50, 9.56, and 9.64 log cfu/g, respectively. In case of *L. casei*, the count was 0.00, 8.40, 8.20 and 8.70 log cfu/g for different treatments in respective order. Hekmat and McMohan (1992) as well as Arunkumar (1993) reported that storage of probiotic ice cream up to 17 weeks showed a decline of *L. acidophilus* to 3x10⁶ cfu/g and *B. bifidum* to 1x10⁷ cfu/g. Results of other studies concord well with our investigations (Nizam and Rai, 2018).

4. CONCLUSIONS

In general, addition of Alphonso pulp or probiotic cultures reduced the fat and total solids content but increased acidity of *Kulfi*. However, no significant effect on protein content in *Kulfi* was observed. Addition of the pulp and probiotic culture improved the sensory quality and overall acceptability score of *Kulfi*. The viability of *L. acidophilus* and *L. casei* added individually or in mixed culture in *Kulfi* mix increased after incubation and also remained in viable form even after storage of the finished product at -18 to -20°C for 24 h.

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