© 2017 IJSRST | Volume 3 | Issue 8 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Standardization of Technology for Preparation of Functional Frozen *Misti Dahi*

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ABSTRACT

The present study aims to standardize the technology for the preparation of functional frozen Misti Dahi, which was prepared by milk using skim milk powder in various ratios, viz. 11% SNF (Mix1), 12% SNF (Mix2) and 13% SNF (Mix 3). In each batch, three levels of sugar was added i.e. Mix 1 (10%), Mix 2 (15%) and Mix 3 (18%). After adding of sugar, the each batch was inoculated with starter culture (combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* in 1:1 ratio) Mix 1 (1%), Mix 2 (2%) and Mix 3 (3%). Then caramel colour was added @ 0.1% (Mix 1), 0.2% (Mix 2) and 0.3% (Mix3). These batches were allowed to incubate at 30^0C, 35^0C and 37^0C temperature. After that storage studied were carried at 0day, 2day, 4 day, 6 day, 8 day and 10 days storage. These samples are stored at -4^0C at refrigeration temperature. The functional frozen Misti Dahi samples of different treatments were analyzed for sensory characteristics (flavor, colour, taste, texture, and overall acceptability) and microbial analysis (Lactic Acid Bacteria, yeast and mould & coliform count). The evaluation process replicated four times. Statistically factorial CRD are applied for analysis of the data. The best sensory quality of functional frozen Misti Dahi was obtained from 13% SNF with 18% sugar and 0.3% colour by the use of 3% inoculums with combination of *Lactobacillus helveticus* and *Streptococcus thermophilus* culture at 37^0C incubation temperature till 6^{th} day of storage period, while Mix 2 was also at par in respect of sensory qualities of functional frozen Misti Dahi. There was no growth of yeast and mould and coliform during storage periods.

Keywords: Functional frozen Misti Dahi, Sensory Quality, Microbial Quality, Storage period.

I. INTRODUCTION

Milk has played a major contribution in the human diet in many different countries across the world. In a predominantly vegetarian population like India, people consume milk as part of their daily diet. About half of India's total milk production is utilized for the preparation of different traditional dairy products.

Fermentation is one of the simplest ways of preserving milk constituents for human consumption. Fermentation process not only increases the shelf life of the product but also adds to taste and improves the digestibility of milk. Dahi is the most popular and oldest fermented milk product of our country, prepared and utilized in various forms in almost all homes. It is an indispensable item of our Indian diet and quite analogous to yoghurt. It is consumed either as a part of the daily diet or as a refreshing beverage (Singh *et al*., 2015).

Misti Dahi is also known as Misti Doi, Payodhi and Lal dahi. It is a traditional sweetened fermented milk product popular in the eastern part of India, notably West Bengal, Bihar and Assam. It is prepared by lactic acid fermentation of sweetened milk. Misti Dahi is regarded as a special dessert on ceremonial occasions both in the rural and urban Bengal. The product is commonly available in earthen pots of different sizes. A good Misti Dahi has a characteristic brown colour, firm consistency, smooth texture and caramelized flavour.

Frozen Misti Dahi is a frozen dessert that combines the flavors and textures of ice-cream. Functional frozen Misti Dahi can improve the general conditions of the body, decrease the risk of some diseases and could even be used for curing some illness.

As the health benefits of these products, manufacturers are continuously investigating value-added. It is an ideal vehicle for the delivery of probiotic organisms in

the human diet. The addition of viable probiotic cultures to frozen Misti Dahi provides the product with additional functionality and value.

II. METHODS AND MATERIAL

The experimental work was carried out in the Animal Science laboratory of Rural Development and Agricultural Production Department, North Eastern Hill University, Tura, Meghalaya, India.

Raw Materials

Milk: Milk was procured from Rural Development and Agriultural Farm, North Eastern Hill University, Tura, Meghalaya, India. Skimmed milk powder and sugar were purchased from the local market of Meghalaya.

Culture: *Lactobacillus helveticus* and *Streptococcus thermophilus* were obtained from the Anand Agricultural University, India.

Mixed Culture: Mixed culture of *Lactobacillus helveticus* and *Streptococcus thermophilus* were propagated in sterile skim milk test tubes by inoculation and incubation at 37^0C. After incubation the culture were stored at 4^0C.

Preparation of functional frozen Misti Dahi

Functional frozen Misti Dahi Mixes composition and formulations are shown in Table 1. The mixes were divided into 3 parts. Mix 1 was standardized to 11% SNF and 10% sugar, Mix 2 was standardized to 12% SNF and 15% sugar and Mix 3 was standardized to 13% SNF and 18% sugar. All the three mixes were heat treated at 80°C for 10min, then cooled to 37^0C - 40^0C. Caramel colour was added at the rate of 0.1% (Mix 1), 0.2% (Mix 2), 0.3% (Mix 3) and yoghurt starter culture was added in different 1% (Mix 1), 2% (Mix 2) and 3% (Mix 3) concentration. The resultant Frozen Sweet Curd was packed in cups (100 ml, then incubated at 30^0C for 0 hour (Mix 1), 35^0C for 6 hour (Mix 2) and 37^0C for 12 hour (Mix 3). The finished products were put in deep freezer at -18°C for hardening for 3 hour before analysis and stored for 10 days.

Milk
↓
Heated to (60-62^{0}C)
↓
Standardization of SNF (11%, 12% and 13% Mix 1, Mix 2 and Mix 3)
↓
Heating (90^{0}C)
↓
Addition of sugar (10%, 15% and 18% Mix 1, Mix 2 and Mix 3)
↓
Holding for 5 minutes
↓
Cooling to (37-40^{0}C)
↓
Addition of caramel colour and flavour (0.1%, 0.2% and 0.3% Mix 1, Mix 2 and Mix 3)
↓
Inoculation with culture (1%, 2% and 3% Mix 1, Mix2 and Mix 3)
↓
Packaging in cups (100ml)
↓
Incubation at 30^{0}C for 0 hr, 35^{0}C for 6 hr and 37^{0}C for 12hr (Mix 1, Mix 2 and Mix 3)
↓
Hardening (-18^{0}C)

Figure 1: Flow diagram for the preparation of functional Frozen Misti Dahi

Sensory Evaluation

The functional frozen Misti Dahi sample was evaluated for the sensory attributes of flavor, color, taste, texture and overall acceptabilty by 5 random identified panelits (Amerine *et al*. 1965).

Microbial Analysis

Lactic Acid Bacterial Count: *Lactobacillus helveticus* and *Strptococcus thermophilus* counts were enumerated in each formulation on 0, 2, 4, 6 and 10 days of storage period. Cultures were enumerated by the pour plate technique. Each sample was serially diluted (1:9) in 0.1% sterilized peptone water. Appropriate dilutions were prepared using the following media: MRS Agar for the enumeration of *Lactobacillus helveticus*; was incubated anaerobically at 37±3°C for 2 days; M-17 agar for the enumeration of *Strptococcus thermophilus.*

Yeast and mould count: Nutrient Agar was used to enumerate yeast and mould count. The first dilutions of functional frozen Misti Dahi samples were taken in duplicate into petriplates and then Nutrient Agar (NA) was added and mixed well. The plates were allowed to solidify. The plates were again over layered with the same agar and allowed to solidify. These plates were incubated at 37ºC for 72 hour and numbers of yeast and mould colonies developed were counted as colony forming units (c.f.u.) per ml.

Presumptive coliform test: The first dilutions of frozen Misti Dahi samples were taken in duplicate into petriplates and then Rose Bengal agar (RBA) was added and mixed well. The plates were allowed to solidify. The plates were again over layered with the same agar and allowed to solidify. These plates were incubated at 37ºC for 24 hour and numbers of coliform colonies developed were counted as colony forming units (c.f.u.) per ml.

Statistical Analysis

All the tests were carried out in triplicate. Significant differences between treatments were tested by analysis of variance (ANOVA) with a level of significance.

III. RESULTS AND DISCUSSION

Sensory evaluation of frozen Misti Dahi:
Sensory evaluation of the 3 mixes of functional frozen Misti Dahi was carried out among 5 panelists, using 9-point Hedonic Scale Method.

Flavor: The highest mean value of functional frozen Misti Dahi flavor was noted in Mix 3 (7.6) followed by Mix 2 (5.9). The least value flavor was computed under Mix 1 (4.5). Zero day storage period gave highest mean value of 7.0 followed by two days 6.8. Whereas least mean value of 4.4 was given by 10 days storage periods (Table 1 & Figure 2). The results were in accordance with the findings of Agarwal *et al.* (2013). However the results were different from those reported by Kosikowski *et al*. (1981).

Colour: The panelists preferred Mix 3 more than Mix 2 and Mix 1. Mix 3 obtained the mean score of Liking of 7.3 followed by Mix 2 (6.4) and Mix 1 (3.6) (Table 1 & Figure 2). The colour intensity mean score of the 3 Mixes were significantly higher. Mix 3 showed the darkest color, then Mix 2 and Mix 1, the lightest. The zero day storage of functional frozen Misti Dahi samples was the highest (6.9) and the lowest was the tenth day (4.5).

Table 1: Mean summary table for flavor, colour, taste, texture and overall acceptability of functional frozen Misti Dahi during different storage periods.

a) Flavor							
Composition	**Storage Periods (day)**						
	0	**2**	**4**	**6**	**8**	**10**	**Mean**
Mix 1	5.7	5.3	5.2	4.4	3.5	2.8	**4.5**
Mix 2	6.7	6.5	6.3	5.8	5.3	4.7	**5.9**
Mix 3	8.8	8.6	8.2	7.6	6.8	5.7	**7.6**
Mean	**7.0**	**6.8**	**6.6**	**5.9**	**5.2**	**4.4**	**6.0**
C.D $_{0.05}$ Composition : 0.11 : S, Period : 0.17 : S and Composition x Period : 0.28 : S							
b) Colour							
Composition	**Storage Periods (day)**						
	0	**2**	**4**	**6**	**8**	**10**	**Mean**
Mix 1	4.9	4.0	3.6	3.3	3.1	2.5	**3.6**
Mix 2	7.6	7.3	6.6	6.4	5.6	4.7	**6.4**
Mix 3	8.3	7.8	7.5	7.2	6.6	6.3	**7.3**
Mean	**6.9**	**6.4**	**5.9**	**5.6**	**5.1**	**4.5**	**5.7**
C.D $_{0.05}$ Composition : 0.14 : S, Period : 0.17 : S and Composition x Period : 0.28 : S							
c) Taste							
Composition	**Storage Periods (day)**						
	0	**2**	**4**	**6**	**8**	**10**	**Mean**
Mix 1	5.4	6.2	6.1	6.3	6.0	4.4	**5.7**
Mix 2	7.7	7.7	7.6	7.4	6.6	5.2	**7.0**
Mix 3	8.9	8.9	8.6	8.3	7.5	6.6	**8.1**
Mean	**7.3**	**7.6**	**7.4**	**7.3**	**6.7**	**5.4**	**6.9**
C.D $_{0.05}$ Composition : 0.14 : S, Period : 0.17 : S and Composition x Period : 0.28 : S							
d) Texture							
Composition	**Storage Periods (day)**						
	0	**2**	**4**	**6**	**8**	**10**	**Mean**
Mix 1	3.1	3.8	3.9	3.9	3.8	3.6	**3.7**
Mix 2	7.6	7.4	7.5	7.2	6.5	5.6	**7.0**
Mix 3	8.7	8.8	8.6	8.4	8.2	7.5	**8.3**
Mean	**6.5**	**6.7**	**6.6**	**6.5**	**6.1**	**5.6**	**6.3**
C.D $_{0.05}$ Composition : 0.34 : S, Period : 0.43 : S and Composition x Period : 0.74 : S							
e) Overall acceptability							
Composition	**Storage Periods (day)**						
	0	**2**	**4**	**6**	**8**	**10**	**Mean**
Mix 1	4.8	4.8	4.7	4.5	4.1	3.3	**4.4**
Mix 2	7.4	7.2	7.0	6.7	6.0	5.1	**6.6**
Mix 3	8.7	8.5	8.2	7.9	7.2	6.5	**7.8**
Mean	**7.0**	**6.8**	**6.6**	**6.4**	**5.8**	**5.0**	**6.3**
C.D $_{0.05}$ Composition : 0.09 : S, Period : 0.11 : S and Composition x Period : 0.20 : S							

Taste: Good quality Misti Dahi has pleasant milky to slight sour taste. The results of taste are shown in Table 1 & Figure 2. The panelists preferred Mix 3 more than

Mix 2 and Mix 1. Mix 3 obtained the maximum mean value of Liking of 8.1. The minimum taste of 5.7 is obtained by Mix 1. The results displayed significant different among the different storage period. The highest taste value 7.6 was computed under second day storage periods, whereas lowest taste value of 5.4 was computed under tenth day storage periods. Similar findings were also observed by Paul *et al.* (2016) where they observed that the taste of the Misti Dahi supplemented with flax lignan were slightly decreased throughout the storage periods.

Texture: Perusal of results makes it clear that the Mix 3 obtained the highest mean score of Liking of 8.3 which was significantly higher than Mix 2 and Mix 1, respectively. The lowest texture of 3.7 was obtained by Mix 1. Ingredients used on the media in the multiplication process might contribute to the high desirable smoothness of Mix 3. Texture scores decreased during storage period. The average scores of texture of functional frozen Misti Dahi under storage periods were computed as 6.5, 6.7, 6.6, 6.5, 6.1 and 5.6 at zero, two, four, six, eight and ten days, respectively, which were significantly, differ from each other (Table 1 & Figure 2).

Overall acceptability: The overall acceptability of functional frozen Misti Dahi was determined on the basis of the average of the total score obtained for different sensory attributes viz. flavor, colour, taste and texture. Overall acceptability of frozen yogurt is affected by milk used for production, the culture, additives and production techniques (Ayar *et al*., 2005). Average overall acceptabilty of functional frozen Misti Dahi are shown in Table 1 & Figure 2. Highest score was obtained by Mix 3 (7.8) followed by Mix 2 (6.6) and Mix 1 (4.4). All the three mixes were statistically significant. The average values of overall acceptability of functional frozen Misti Dahi affected by different storage period. The significantly highest score was obtained by zero day (7.0) and significantly lowest score was obtained by tenth day (5.0) storage period.

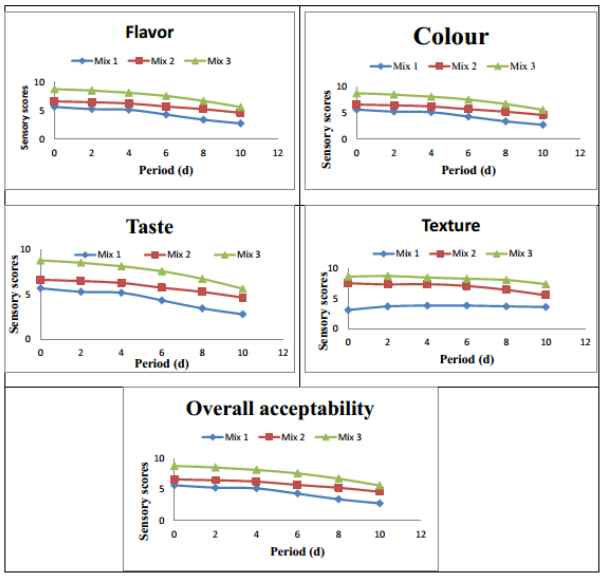

Figure 2 : Sensory evaluation of functional frozen Misti Dahi

Microbiological Analysis

Lactic Acid Bacteria Count:

The dairy products, especially functional frozen Misti dahi are good vehicle to carry probiotics to the human intestinal tract. Consumption of probiotic bacteria via dairy food product is an ideal way to re-establish the intestinal micro-flora balance. Table 2 & Figure 3 showed lactic acid bacteria counts in the three mixes of functional frozen Misti dahi. The average *Lactobacillus helveticus* counts of frozen Misti Dahi were 7.11 log cfu/ml (Mix 1), 8.42 log cfu/ml (Mix 2) and 8.78 log cfu/ml (Mix 3). Whereas the average *Streptococcus thermophilus* counts of frozen Misti Dahi were 7.42 log cfu/ml (Mix 1), 8.44 log cfu/ml (Mix 2) 8.83 log cfu/ml (Mix 3).

From the Table 2, it was observed that the *Lactobacillus helveticus* and *Streptococcus thermophilus* counts were continuously decreases with storage periods. The maximum counts of *Lactobacillus helveticus* 8.27 log cfu/ml were obtained by zero day storage periods. However the minimum *Lactobacillus helveticus* counts of 7.84 log cfu/ml were obtained in ten days storage periods.

The viability loss of *Streptococcus thermophilus* was also observed significant during the storage period. The maximum *Streptococcus thermophilus* counts of 8.38

log cfu/ml was obtained in zero day storage periods and the minimum counts of 8.03 log cfu/ml was obtained in 10 days storage periods. Lopez *et al*. (1998) observed only a slight decline in three batches (pH= 4.32, 5.09 and 5.53) of commercial frozen yoghurt stored at -23^{0}C for 1 year. Davidson *et al*. (2000) reported that the initial bacterial counts recovered from the frozen yogurt for *Lactobacillus bulgaricus* and *Streptococcus thermophilus* did not decline over the 11-wk storage period.

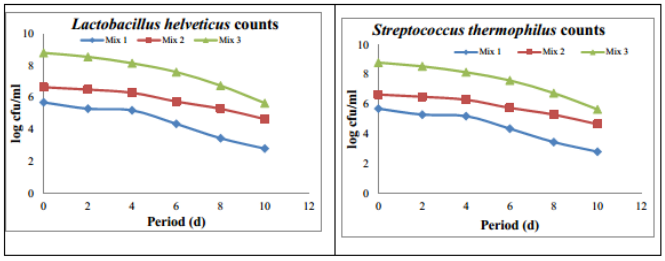

Figure 3 : Changes in viability of *Lactic Acid Bacteria* in functional frozen Misti Dahi

Table 2: Mean summary table for Lactic Acid Bacteria counts at different storage periods

***Lactobacillus helveticus* count**							
Composition	**Storage Periods (day)**						
	0	**2**	**4**	**6**	**8**	**10**	**Mean**
Mix 1	7.33	7.22	7.17	7.12	7.12	6.72	**7.11**
Mix 2	8.65	8.59	8.59	8.35	8.18	8.16	**8.42**
Mix 3	8.83	8.83	8.82	8.80	8.72	8.65	**8.78**
Mean	**8.27**	**8.21**	**8.19**	**8.09**	**8.01**	**7.84**	**8.10**
C.D $_{0.05}$ Composition : 0.09 : S, Period : 0.13 : S and Composition x Period : 0.22 : NS							
***Streptococcus thermophilus* count**							
Composition	**Storage Periods (day)**						
	0	**2**	**4**	**6**	**8**	**10**	**Mean**
Mix 1	7.53	7.53	7.52	7.37	7.31	7.27	**7.42**
Mix 2	8.72	8.69	8.68	8.26	8.18	8.12	**8.44**
Mix 3	8.89	8.89	8.88	8.83	8.76	8.71	**8.83**
Mean	**8.38**	**8.37**	**8.36**	**8.15**	**8.08**	**8.03**	**8.23**
C.D $_{0.05}$ Composition : 0.07 : S, Period : 0.10 : S and Composition x Period : 0.18 : S							

Yeast and Mould count:

The yeast and moulds are one of the most important group of spoilage microflora in acidified dairy products, capable of reducing their shelf life, even under refrigerated storage. All the Mixes were subjected to yeast and moulds test and it was found to be negative yeast and moulds counts. No colony was grown in zero to ten days of storage.

Coliform count:

All the Mixes were subjected to coliform test and it is found to be negative coliform test. This indicated that the functional frozen Misti Dahi treatments were free from gas producing organisms. This was possible due to strict sanitary condition observed between during each step of manufacture. Contamination with the coliform organism is a common problem is the industry and they are completely undesirable in any products. The absence of coliform organism indicates that proper care was taken during processing to avoid post processing contamination and product is of food quality. This work is in similarity with Agarwal and Prasad (2013), where they reported that there was no evidence of coliform in low-fat frozen yoghurt.

IV. CONCLUSION

There is growing consumer interest in food products that can provide health benefits. The functional frozen Misti Dahi might be a good source for probiotic cultures, due to their composition. Frozen storage of the product has little or no effect on culture survival, and bacterial cultures remained at levels sufficient to offer the suggested therapeutic effects. Supplementation with probiotic bacteria has little effect on flavor or compositional characteristics of functional frozen Misti Dahi. Present study showed the potential in the development of functional frozen Misti Dahi products which provide the advantage of viable probiotic in the products. The finished products contained high levels of protein and had good flavor, colour taste and body characteristics.

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