Effect of levels of starter culture and varieties of packaging materials on the quality characteristics of enriched misti dahi

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ABSTRACT

The research was undertaken to access the effect of starter culture on the qualitative characteristics of whey protein enriched misti dahi through incorporation of whey protein concentrate @ 2.0 % whey protein concentrate and fruit juice (apple and orange - 1:1) @ 15.0 % by weight. The thermophilic starter cultures were added @ 1.0, 2.0 and 3.0 % level. Incorporation of starter culture @ 2.0 % rate yielded product having acceptable sensory attributes. Misti dahi packaged in polyethylene teraphthalate (PET) cups could be stored for longer period (i.e. 15 days) as compared to use of polystyrene, polypropylene cups or even earthen pots, when stored under refrigerated (4±2 °C) condition.

Key words: Culture, Keeping quality, Misti dahi, Packaging materials.

INTRODUCTION

Dahi is an Indian fermented milk product known for its refreshing taste, palatability and therapeutic values. Since ancient times, dahi has received its importance as dietary adjunct. The popularity of dahi is not only due to its refreshing taste and palatability but also due to its scientifically proven role as a nutritious milk product. Dahi for direct consumption may be further classified into various types depending upon the type of milk used and addition of sugar etc. They are sweet dahi and sour dahi.

Misti dahi also known as pyodhi, lal dahi, sweetened dahi and mishti doi, has been a well established dessert in the eastern parts of India. It is a popular dessert in West Bengal, where it is served with the meal accompanied by dried sweets. According to ancient Bengali literature, milk meant for curdling was first thickened by boiling before adding caramelized palm jaggery, cane jaggery or sugar and then allowed to set as curd to yield misti dahi. This was often done in an earthen vessel, which contributes a characteristic earthy flavor and to some extent helps in absorbing free whey at the end of the fermentation process (Aneja et al., 2002).

The nutritional (lecithin, sphingomyline) and functional properties (like stabilizer, imitator in fermented foods) of whey protein concentrate (WPC) are well documented and fortification of cow or buffalo milk with fruit pulp improves the nutritional as well as therapeutic values (like antioxidant and antifungal) of dahi (Kale, 2011).

The nourishment of fruit juices and addition of whey protein concentrate misti dahi will increase the total amount of nutrients in the misti dahi; moreover, the consumption of fruit juices and whey protein concentrate will add value to misti dahi. Packaging plays a significant role, in increasing shelf life of fermented milk products.

Generally mixed culture of Streptococcus lactis, Streptococcus thermophilius, Lactobacillus bulgaricus and Lactobacillus plantarum are used for making different types of dahi. The amount of culture necessary depends mainly on season of the year due to the variations in ambient temperature. In summer 1 to 2 % and in winter 5 to 10 % by volume of culture are required for optimum coagulation. Together with culture, 10 to 12 % sugar is added for making sweet dahi but no sugar is required for making sour dahi. Fruit or flavoured dahi is prepared by using different fruit juice or added flavour together with culture and sugar.

Polypropylene (PP), Polystyrene (PS) and Polyethylene teraphthalate (PET) containers are clean in appearance; give a good shining to look. They are light in weight and unbreakable. Dahi and Misti dahi are packaged and sold in local market by halwais, dhabas and mini dairies in earthen pots. Advantages of using earthen pots are that dahi packaged in earthen pots has a firm body and these packs are eco-friendly and easily degradable after disposal (Matkar and Khedkar et al., 2008). Due to its delicious taste and firm body, misti dahi has good market potential in all parts of the country. Keeping this in view, an attempt has been made in this investigation to develop the enriched misti dahi with the following objectives. To optimize the effect of various levels of starter cultures on the sensory quality of enriched misti dahi and to study the shelf stability of enriched misti dahi.

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MATERIALS AND METHODS
Raw materials and ingredients
Whole milk: Raw milk (Mixed) procured from Students Experimental Dairy Plant (SEDP) was used for the preparation of misti dahi.
Sugar: Good quality sugar procured from the local market was used in the preparation of misti dahi.
Whey Protein Concentrate- Fresh spray dried Whey Protein Concentrate having 80% protein was procured from Mahaan Proteins Ltd., New Delhi.
Fruit juices (apple and orange juices) - Apple and orange juices of Tropicana brand were procured.
Starter cultures - Cultures such as Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus in the form of freeze dried direct vat (FD-DVS) was obtained from Chr. Hansen Laboratories, Copenhagen, Denmark through their distributor at Bangalore, India.

The starter culture maintained in the Postgraduate Laboratory, Dairy Microbiology Department, Dairy Science College, KVAFSU, and Bengaluru was used in this study. Mixed starter culture of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus in the ratio of 1:1 was used in the preparation of enriched misti dahi. The stock cultures were maintained in yeast glucose chalk litmus milk and sub cultured once in 21 days, whereas the working cultures were maintained in sterile skim milk.

Packaging materials- Packaging materials such as Polypropylene (PP), Polystyrene (PS) and Polyethylene terephthalate (PET) cups and Earthen pots of 100 ml (For trial purpose) were used for packaging of misti dahi. Such packaging materials were procured from Ramdev Plastics, Bengaluru.

Optimizing the level of starter culture in the preparation of misti dahi: Milk was obtained from Student Experimental Dairy Plant, Karnataka Veterinary Animal and Fisheries Sciences University (KVAFSU) of Hebbal, Bengaluru. Optimization of level of starter culture was carried out by using them at levels of 1, 2 and 3% in the preparation of misti dahi. Modification in the standardized process adopted by Singh (2006) was done to optimize the level of starter culture to be used. The levels of WPC and fruit juice were optimized based on research carried out by Soumya et al., 2017.

Chemical analyses of misti dahi: Fat content in misti dahi samples was determined by Gerber method (BIS 1981), Total Solids (TS) of milk, misti dahi samples was determined by gravimetric method (BIS 1981). Titratable acidity of the same was measured (BIS 1981). pH was measured using a digital pH meter (Elico Pvt.Ltd., Mumbai, India) at 25°C. About 20 ml of representative samples were used for pH measurement.

Microbial analysis of misti dahi: Coliform count and Yeast and Mould count were analyzed as per BIS (1981).

Sensory evaluation of misti dahi: Misti dahi samples were provided to a panel of seven judges for sensory evaluation. Each judge was supplied with 9 Point Hedonic Scale (Annexure-1) for colour and appearance, body and texture, flavor and overall acceptance (not appearance). The samples were code numbered to avoid identification and bias (Dharam and Gupta 1985).

Statistical analysis: The results are the average of three replications which was statistically analyzed using ANOVA technique (Two way ANOVA with replications) for one way analysis with independent samples (Zar, 2003).

RESULTS AND DISCUSSION
Optimizing the level of starter culture based on the sensory quality of misti dahi: The effect of three levels of starter culture on the sensory quality of misti dahi is presented in Table 1. The sensory scores awarded for overall acceptability of misti dahi was 8.31 (for control) as against 7.56, 7.67 and 7.47 for misti dahi made using 1.0, 2.0 and 3.0% starter culture respectively. Misti dahi prepared using 2.0% starter culture scored highest average sensory score as compared to those prepared using 1.0 and 3.0%, and was found significantly superior. The least score of 7.47 was associated with misti dahi prepared using 3.0% starter. Statistically there was non-significant difference between control and experimental samples with respect to overall acceptability. Therefore in all the subsequent experiments misti dahi was prepared using starter culture @ 2.0% by weight of milk only. These results are in conformity with the findings of Akter et al. (2010) for misti dahi prepared using different levels of starter culture; 2.0% starter culture along with 10.0% sugar yielded misti dahi having best sensory scores.

Effect of packaging materials on the sensory quality of misti dahi: The fresh control and WPC enriched misti dahi samples (0 day) packed in Polystyrene (C), Polyethylene terephthalate cups (P1), Polypropylene (P2) cups and earthen pots (P3), were subjected to sensory evaluation. The results pertaining to the effect of different packaging materials on the sensory quality of misti dahi are presented in Table 2.

The physical appearance of misti dahi plays an important role in consumer acceptability. The overall acceptability scores were found to be 7.83, 8.31, 7.76 and 7.63 for product packaged in C, P1, P2 and P3 respectively. P2 had the highest sensory score with respect to control, when compared to P1 and P3. The type of packaging materials had a significant effect (P ≤ 0.05) on sensory quality of control and enriched misti dahi. The samples stored in PET cups were awarded highest appearance score (i.e. 7.89 out of 9.00) while control packaged in PET product had a score of 8.11. Similarly for body and texture, the control and the
Table 1: Effect of levels of starter culture on the sensory quality of enriched misti dahi.

| Level of starter culture (%) | Colour and Appearance | Flavour | Body and Texture | Overall acceptability |
|-----------------------------|-----------------------|---------|------------------|----------------------|
| Control*                    | 8.11a                 | 8.17a   | 8.19a            | 8.31a                |
| 1.0                         | 7.61a                 | 7.56a   | 7.56a            | 7.56a                |
| 2.0                         | 7.78a                 | 7.56a   | 7.64a            | 7.67a                |
| 3.0                         | 7.53a                 | 7.25a   | 7.24a            | 7.47a                |
| CD(P<0.05)                  | NS                   | NS      | NS               | NS                   |

%) As per directions from the supplier.

Note: All values are average of three trials, NS – Non significant, Similar superscripts indicate non – significance at the corresponding critical difference, Control – Plain Misti dahi, Treated Misti dahi contains 2% WPC and 15% fruit juice blend.
product packaged in PET cups were associated with higher average score of 8.17 and 7.78 out of 9.00 respectively. The developed products packed in PET cups had the highest scores for overall acceptability of 7.83 as against control product which had score of 8.31. Similar observations have been reported by Reddy (2011) for low fat yoghurt where he used PP, PET and Earthen pots, incorporated with pectin, WPC, essential oils and nisin.

Table 2: Effect of packaging materials on the sensory quality of fresh misti dahi.

| Samples in different packages | Sensory score for attributes (out of 9.0) | Colour and Appearance | Body and Texture | Flavour | Overall acceptability |
|------------------------------|------------------------------------------|-----------------------|------------------|---------|----------------------|
| Control                      |                                          | 8.11*                 | 8.17*            | 8.19*   | 8.31*                |
| P1                           |                                          | 7.89b                 | 7.78b            | 7.82b   | 7.83b                |
| P2                           |                                          | 7.79b                 | 7.86b            | 7.69b   | 7.76b                |
| P3                           |                                          | 7.73b                 | 7.50b            | 7.67b   | 7.63b                |
| CD(P ≤0.05)                  |                                          | 0.07                  | 0.10             | 0.06    | 0.04                 |

Note: All values are average of three trials. Similar superscripts indicate non-significance at the corresponding critical difference. Treated samples of misti dahi was added with 2% WPC and 15% fruit juice blend, C- Control misti dahi was packed in polystyrene cups, P1 - misti dahi packed in PET cups, P2 - misti dahi packed in PP cups, P3 - misti dahi packed in earthen pots.

Table 3: Effect of packaging materials on the physico-chemical quality of fresh misti dahi stored at refrigeration temperature (4±1°C).

| Sample | Storage period (days) |
|--------|------------------------|
|        | 0   | 3   | 6   |
| C      | Total Solids | Acidity | Fat | pH | Total Solids | Acidity | Fat | pH | Total Solids | Acidity | Fat | pH |
|        | 30.04a        | 0.70a   | 5.02a | 4.56a | 30.12a | 0.71a   | 5.02a | 4.55a | 30.24a | 0.76a | 5.03a | 4.43a |
| P1     | 30.26b        | 0.71b   | 5.11b | 4.55b | 30.31b | 0.73b   | 5.11b | 4.48b | 30.35b | 0.79b | 5.12b | 4.36b |
| P2     | 30.20b        | 0.71b   | 5.13b | 4.55b | 30.36b | 0.75b   | 5.13b | 4.46b | 30.58b | 0.82b | 5.13b | 4.31b |
| P3     | 32.92b        | 0.75b   | 5.54b | 4.46b | 35.65b | 0.77b   | 5.54b | 4.43b | 40.58b | 1.00b | 5.64b | 4.20b |
| CD     | 0.21          | 0.003   | 0.002 | 0.018 | 0.18    | 0.004   | 0.02  | 0.02  | 0.14    | 0.006 | 0.02  | 0.018 |

Note – All the values are average of three trials C – Control misti dahi packed in polystyrene cups
P1 - Misti dahi packed in PET
P2 - Misti dahi packed in Polypropylene cups
P3 - Misti dahi packed in earthen pots

Table 4: Effect of packaging material on the microbiological quality of control and experimental misti dahi stored at refrigeration temperature.

| Sample | Microbial count (log_{10} cfu/g) in misti dahi during storage period (days) |
|--------|-------------------------------------------------------------------------|
|        | 0   | 3   | 6   | 9   | 12  | 15   |
| C      | Coli | Y&M | Coli | Y&M | Coli | Y&M | Coli | Y&M | Coli | Y&M | Coli | Y&M |
| P1     | NIL | NIL | NIL | 0.19 | 0.23 | 0.45 | 0.32 | 0.64 | 0.61 | 0.90 | 0.87 | 1.48 |
| P2     | NIL | NIL | NIL | 0.20 | 0.29 | 0.56 | 0.57 | 0.84 | 0.96 | 1.50 | Spoiled |
| P3     | NIL | NIL | NIL | 0.28 | 0.58 | 0.93 | 0.93 | 1.52 | Spoiled |
| CD     | -   | -   | -   | 0.04 | 0.01 | 0.02 | 0.02 | 0.04 | 0.03 | 0.02 | -   | -   |

Note – All the values are average of three trials
Effect of packaging materials on the sensory quality of misti dahi stored at refrigeration temperature (4±2°C).

Upon storage there was loss of moisture (initial and final moisture in the product upon storage) in all the samples with simultaneous increase in fat content. The findings are in accordance with Sonawane and Pawar (2007) who noted significant (P ≤0.05) moisture loss in shrikhand samples during storage at refrigeration temperature irrespective of the type of packaging materials used. Similar studies regarding effect of packaging materials on the acidity and pH of low fat yoghurt by Reddy (2011) revealed that the acidity of the product increased during storage irrespective of the packaging material (PP and PET). The extent of increase in acidity and decrease in pH was comparatively slower for samples stored under refrigeration condition compared to that stored at room temperature. This may be due to the products higher Oxygen Transmission Rate (OTR) and Water Vapour Transmission Rate (WVTR) Kumar and Srinivasa, (1983). Thus, the rate of increase in acidity and rancidity may be attributed due to increase in the microbial count and lactase enzyme.

Effect of packaging materials on the microbial quality of misti dahi stored at refrigeration temperature (4±1°C): The control misti dahi packed in polystyrene (C) and experimental misti dahi packed in Polyethylene teraphthalate (P), Polypropylene (P2) and earthen pot (P3) cups and earthen pot (P3) were stored at refrigeration temperature and the stored samples analyzed for microbial quality at 3 days interval. The change in the microbial quality during refrigerated storage (4±1°C) of misti dahi samples are tabulated in Table 4.

The coliforms and yeast and mold were absent in freshly prepared control as well as all the experimental samples. At 3rd day of storage coliform count were still absent in C, P1 and P samples, but P3 sample had coliform count of 0.14 log_{10} cfu/g. On 3rd day of refrigerated storage, samples C, P1, P2 and P3 showed yeast and mold count of 0.19, 0.20, 0.28 and 0.96 log_{10} cfu/g respectively; such counts increased during further storage but were well within FSSA standards of Dahi. The shelf life of P3 was 9 days, beyond which period surface discolouration and mold growth was observed. On 12th day of storage, P3 became unacceptable and on 15th day of storage P became unacceptable. At same period, C registered coliform and yeast and mold count of 0.87 and 1.48 log_{10} cfu/g respectively. On 16th day, C sample became unacceptable due to perception of off-flavour. Similar findings have been reported by Rao (2009) regarding the effect of packaging materials at room and refrigeration conditions on the microbial quality of low calorie probiotic misti dahi.

Effect of packaging materials on the sensory quality of misti dahi stored at refrigeration temperature (4±1°C)

Effect of different packaging materials on the sensory quality of control and experimental misti dahi stored at refrigeration temperature 4±1°C is presented in Table 5.
The samples were subjected to sensory evaluation at an interval of 3 days. The sensory score for all the samples decreased progressively during storage period. There was a significant (\( P \leq 0.05 \)) effect on all of the sensory attributes of misti dahi samples. \( P_1 \) secured significantly (\( P \leq 0.05 \)) higher scores than \( P_2 \) and \( P_3 \); the former sample showed least whey separation. \( P_2 \) and \( P_3 \) samples were unacceptable at 9th and 12th day of storage respectively due to surface discoloration, off odour and higher level of whey separation. On 15th and 16th day, PET and control became unacceptable owing to development of off flavour. The shelf life of misti dahi may be influenced by an increase in the microbial count and lactase enzyme Dager and Ali (1985) reported that destruction of yoghurt bacteria increased progressively with increase in heat treatment, because of slower production of acid during storage take place in all the packaging materials. The current findings were in agreement with the findings of Ashok (1990) who reported that, shelf life of the sweet cream buttermilk based yoghurt drink could be extended up to 2 weeks under refrigeration condition and 2 days at room temperature.

**CONCLUSION**

Misti dahi is a fermented milk product prepared by using selective cultures under controlled condition. Enriched misti dahi developed to exert therapeutic and nutritional benefits, to meet the requirement of vulnerable population. Enriched misti dahi was developed by incorporation of 2.0 per cent whey protein concentrate, fruit juice (apple and orange 1:1) incorporated at 15 per cent and culture at 2.0 per cent. With view to preserve value added enriched misti dahi, different packaging material was used. The developed product packed in PET had secured highest sensory scores and had shelf life of three days at room temperature (30±1°C) and 12 days at refrigeration temperature (4±1°C) without affecting sensory attributes with respect to control. Thus the developed enriched misti dahi can be commercialized under large scale.

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