Compositional and Microbiological Changes in Dahi Added with Strawberry Polyphenols Extract and Probiotic Lactobacillus rhamnosus GG

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Abstract

In the present study strawberry polyphenols extract fortified dahi along with the usage of low calorie sweetener aspartame @ 65 ppm was fermented with mesophillic mixed dahi culture (NCDC 167) and Lactobacillus rhamnosus G. Prepared Dahi samples were assessed for compositional and microbiological changes at refrigeration temperature (4°C). Compositional and microbiological parameters of control (without addition of polyphenols extract) and polyphenols fortified dahi were observed to be non significantly (p<0.05) different.

Keywords: Lactobacillus rhamnosus, Flavonoids, Procyanidins, Phenolic acid

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Introduction

Polyphenolic compounds viz. flavonoids, procyanidins, phenolic acid and stildenes are the major important bioactive components of vegetable and fruits. These phenolic compounds have several biofunctional properties such as antioxidant and ACE inhibitory (Sikora et al., 2008). The inclusion of phenolic compound in diet of human is better alternative of usage of synthetic drug without imparting any side effects. As use of synthetic drugs are associated with many ill effects. So there is requirement to incorporate the individual diet with natural bioactive compounds. Among all chronic diseases hypertension and diabetes are commonly occurring metabolic diseases. These are the world foremost chronic diseases.

Current scenario has a greater place for functional foods like functional fermented milk and milk products. Dahi is yoghurt like product having wide consumption level in India at every household. Therefore incorporation of polyphenolic compounds like strawberry polphenols extract either in puree or concentrate form can be better way to deliver these bioactive functional ingredients. Strawberry polyphenols incorporation along with the probiotic culture in fermented dairy products would further enhance the
functionality of the product. Hence current study was aimed with objective of compositional and microbiological evaluation of polyphenols fortified dahi fermented with probiotic culture *Lactobacillus rhamnosus GG*.

**Materials and Methods**

Fresh raw milk was obtained from the Livestock herd of National dairy research Institute, Karnal, Haryana. Strawberry pulp was procured from the M/S delta Nutritive Pvt. Ltd., Mumbai. Starter culture mesophilic mixed dahi culture and *Lactobacillus rhamnosus GG* were procured from National Collection of Dairy Cultures, National dairy research institute, Karnal, Haryana. All the other chemicals were purchased from the standard supplier.

**Starter culture and probiotic culture procurement**

Mesophilic homofermentative starter cultures NCDC 167 (containing *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *diacetylactis*, *Lactococcus lactis* ssp. *cremoris* along with *Leuconostoc* spp.) and Lactobacillus rhamnosus GG (probiotic culture) were collected from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal, India. The cultures were propagated at 30°C for 24 hr. and 37°C for 24 hr. in M-17 and MRS broth. Prior to preparation of dahi, the cultures were activated three times in sterilized skimmed milk.

**Analysis of strawberry polyphenols extract**

Strawberry polyphenols extract was prepared as per the methodology developed by Cossu *et al.*, 2009. Then the prepared aqueous extract was analyzed for the total phenolic content using Flin-Ciocalteu method of Zhang *et al.*, 2006. A calibration curve of gallic acid (0-120 µg/ml) was prepared and obtained results were expressed as gallic acid equivalent (GAE)/ml.

**Preparation of low calorie polyphenol fortified dahi**

Compositional parameters were standardized by using the protocol of De (2006). Strawberry polyphenols fortified dahi was prepared as per methodology developed by Richa *et al.*, 2012 with modifications. Milk was fermented with equal proportion of mesophilic mixed dahi culture and *Lactobacillus rhamnosus GG* @1%.

**Compositional and microbiological analysis**

Strawberry polyphenols fortified dahi was assessed for total solids content by procedure described in Directorate general of health services ministry of health and family welfare government of India, New Delhi, 2005. Fat content was estimated by Mojonnier extraction methodology (AOAC, 2000). Protein content was determined as per method ISO 8968-11; 2014.

Water holding property was determined according to method described by Remeuf *et al.*, 2003.

**Microbiological analysis of strawberry polyphenols extract fortified dahi**

Prepared control and polyphenol extract fortified dahi was assessed for Lactococci, Lactobacilli, coliform counts, yeast and mold during storage period of 3 weeks at refrigeration temperature 4°C.

**Lactococci and lactobacilli counts determination**

Lactococci and lactobacilli counts of control and polyphenols fortified dahi were estimated according to procedure described in
Laboratory manual – Method of analysis of milk and milk products (MIF, 1959).

Coliform count estimation

Coliform counts in control and strawberry polyphenols extract fortified dahi were estimated using pour plate method described by Hought by et al., (1992) Colonies with dark red coloration were counted and expressed as log cfu per gm of sample.

Statistical analysis

Data obtained was statistically analyzed in MS excel software at 5 % level of significance. Dat are presented as mean ± standard deviation. Compositional analysis was analyzed by one way ANOVA and storage study data was analyzed by two way ANOVA.

Results and Discussion

Dahi preparation

To prepare low calorie polyphenol fortified dahi milk was fermented with standard dahi culture mesophillic mixed dahi culture (NCDC 167) and probiotic culture Lactobacillus rhamnosus GG in 1:1. Curd was settled in 10 hrs with achieving the acidity corresponding to 0.81% lactic acid and pH 4.8. Set curd was stirred with low speed hand blender and added with 500 ppm polyphenol extract. Aspartame as low calorie sweetener was added @65 ppm as previously reported by Singh et al., 2012. Aspartame was added after fermentation, as addition before fermentation lead to decomposition during fermentation process (YU et al., 2010).

Compositional analysis of polyphenol fortified dahi

The chemical composition of control and strawberry polyphenols fortified dahi as displayed in Table 1 was did not differ significantly (p<0.05).

Changes in pH and acidity

Results displayed in table 2 showed the significant difference (p<0.05) in acidity and pH of control and polyphenols incorporated dahi. Significant (p<0.05) higher acidity of polyphenols fortified dahi might be due to acidic nature of polyphenol extract i.e pH 3.57.

Water holding capacity

Similarly non significant difference (p>0.05) was observed in water holding capacity of control and polyphenol fortified dahi (Table 2).

Changes in pH and acidity during storage

Changes in the pH and acidity of control and polyphenol fortified dahi are presented the figure 1 and 2.

Addition of polyphenols in dahi resulted in lowering of pH 4.6 and increase in acidity 0.99% LA in fresh dahi preparation on 1st day in comparison to control dahi (pH 4.8). During storage period pH was observed to be decreased to 4.3 and increase in acidity to 1.4% as lactic acid was observed on 21st day storage. Faster development of acidity was observed in the polyphenol fortified dahi than control dahi. These changes in acidity and pH in polyphenol fortified dahi might be due to increased activity of starter culture or also biochemical changes made by lactic acid bacteria.

Water holding property

Changes in water holding property of control and polyphenol fortified dahi at refrigeration temperature are presented in Table 3.
Significant decrease in water holding capacity of control and polyphenol fortified dahi was observed on 7th day and thereafter remained unchanged till 18th day of storage period. On 21st day also decrease in the water holding property was observed. This might be the reason of increase in acidity during storage period. Control and Strawberry polphenols extract fortified dahi exhibited the similar compositional attributes except pH and acidity. Hence dahi fortified with strawberry polyphenols extract along with probiotic microorganism can serve as potential function food.

Table 1 Compositional parameters of control and strawberry polyphenols fortified dahi

| Parameters (expressed as %) | Control Stirred Dahi | PP Stirred Dahi |
|-----------------------------|----------------------|-----------------|
| Total solids                | 15.46±0.005         | 15.50±0.01      |
| Fat                         | 1.52±0.008          | 1.51±0.008      |
| Protein                     | 4.98±0.30           | 4.95±0.032      |
| Ash                         | 1.085±0.001         | 1.084±0.001     |

Mean ± S.D, n=3, Means with different superscript (A, B) in each column and row (a,b) differ significantly (p<0.05) significantly from each other.

Table 2 Physico-Chemical analysis of polyphenol fortified dahi

| Parameters                        | Control Dahi     | Polyphenol fortified Dahi |
|-----------------------------------|------------------|----------------------------|
| Acidity (as % lactic acid)        | 0.82±0.008       | 0.96±0.01                 |
| pH                                | 4.83±0.03        | 4.94±0.029                |
| Water Holding Capacity (%)        | 94.82±0.014      | 94.85±0.005               |

Mean ± S.E, n=3
Means with different superscript (A,B) in each column and row (a, b) differ significantly (p<0.05) from each other

Table 3 Changes in water holding property of control and polyphenol fortified dahi during storage

| Storage period (Days) | Water Holding Capacity (%) |
|-----------------------|---------------------------|
|                       | Control Dahi              | PP- Dahi                  |
| 1st                   | 94.82±0.018              | 94.84±0.020               |
| 4th                   | 94.82±0.018              | 94.94±0.029               |
| 7th                   | 93.03±0.026              | 93.05±0.02                |
| 11th                  | 92.89±0.014              | 92.93±0.026               |
| 14th                  | 92.85±0.021              | 92.95±0.02                |
| 18th                  | 92.63±0.43               | 92.47±0.136               |
| 21st                  | 90.73±0.12               | 90.46±0.20                |

Mean ± S.E. n=3
Means with different superscript (A, B, C, D) in each column differ significantly and in each row (a) differ significantly (p<0.05) significantly from each other.
**Fig.1** pH changes in control and polyphenols fortified dahi during storage

![](image1)

**Fig.2** Acidity changes in control and polyphenols fortified dahi during storage

![](image2)

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