ROLE AND VIABILITY OF PROBIOTIC CULTURES IN COW MILK DAHI

D.B. Sakore, P.T. Dhole, K.D. Chavan* and B.K. Pawar

Dept. of Animal & Dairy Science,
Mahatama Phule Krishi Vidyapeeth, Rahuri – 413 722, India.

ABSTRACT

The studies on preparation of cow milk dahi by using probiotic cultures viz., Lactobacillus acidophilus (T1) and Lactobacillus delbrueckii sub sp. bulgaricus (T2) alone as well as in combination their of i.e. T1, T2 and T3. The control dahi (LF-40) contained on average total solids 12.75, fat 4.08, protein 3.22 and acidity 0.75 % LA. The dahi prepared by using Lb. acidophilus (T1) and Lb. delbrueckii sub sp. bulgaricus (T2) alone, contained total solids 12.82 per cent and 12.80 per cent, fat 4.02 per cent and 0.91 per cent, respectively. Dahi (T3) made by using Lb. acidophilus + Lb. delbrueckii subsp. bulgaricus contained on an average total solids 12.81, fat 4.08, protein 3.27and acidity 0.99 per cent lactic acidity. Dahi (T3) prepared by using combination of Lb. acidophilus and Lb. delbrueckii subsp. bulgaricus showed higher viable lactobacilli count (77 x 10^7 c.f.u./gm) than T2 (47 x 10^7 c. f. u./gm) and T1 (30 x 10^7 c f.u./gm) samples respectively. The sensory score for aroma of the dahi sample T3 showed maximum (7.90) and which was at par with control (T0) dahi and T1 dahi samples.

INTRODUCTION

Fermented milks are known throughout the world for their taste, nutritive value and therapeutic properties. Dahi is a Indian fermented milk product known for its refreshing taste, palatability and therapeutic values (Madan Lal et al., 1980). 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. In Indian sub-continent the conversion of milk in every household by souring with the left over of previous day sour milk has been common practice ever since the Aryan inhabited land.

The lactic acid bacteria involved in preparation of fermented milks include selected species of lactobacilli, streptococci, leuconostoc. These microbes have also proved their worth in developing nutritional and therapeutic properties in fermented milks. The presence of lactobacilli in gut helps in maintaining better health of consumers.

Now, the consumers are increasingly interested in their personal health and expect the food that they eat to be healthy or even capable of preventing illness. Gut health in general has shown to be the key sector for functional foods in the world. The yoghurt market is well established but the key growth sector recently has been probiotic yoghurt, drinks and foods (Sandholma et al., 2002). The consumption of fermented milk containing lactobacilli have improved lactose digestion which aids lactose intolerant individuals (Gilliland et al., 1985), decrease in serum cholesterol levels, contribute to the maintenance of the enterohepatic circulation of bile acids through deconjugation of bile acids (Gilliland and Kim, 1984), increases vitamin B content of food (Renner, 1986) increases diabetes weight gain in test suggesting a more efficient utilization of nutrients (McDonough et al., 1982). Dairy products cultured with lactobacilli reported to possess anti carcinogenic properties (Hosono, 2002).

Considering the above facts and importance of cow milk, fermented milk products and probiotic cultures in the human diet, the present investigation was planned.

MATERIAL AND METHODS

Collection of milk samples

Fresh crossbred cow milk samples were obtained form Institutes Dairy From in the morning hours.

*College of Agriculture, Pune – 411 003, India.
Starter culture, its propagation and maintenance

Freeze dried pure cultures of LF-40, Lactobacillus acidophilus and Lactobacillus delbrueckii subsp. bulgaricus were obtained from NDRI, Karnal, Haryana. These cultures were maintained separately in sterilized reconstituted skim milk. The 10 ml reconstituted skim milk test tubes were prepared and they were sterilized. The sterilized skim milk test tubes were separately inoculated with these cultures and incubated at 37°C for 8 hr and thereafter stored at 5°C in order to keep these cultures active, they were propagated once in a week.

Preparation of dahi

The dahi samples were prepared by using the procedure described by De (1985) with some minor modifications.

Treatment details

T₀ : Dahi samples prepared using LF-40 starter culture
T₁ : Dahi samples prepared using lactobacillus acidophilus culture
T₂ : Dahi samples prepared using lactobacillus delbrueckii sub sp. bulgaricus culture
T₃ : Dahi samples prepared using lactobacillus acidophilus and lactobacillus delbrueckii sub sp. bulgaricus culture

Chemical analysis

Chemical analysis of milk and dahi samples

The milk samples were analysed for fat and protein content as per BIS (1981). The dahi samples prepared under experimental treatment were analysed for total solids, fat, protein and acidity as per the procedure described in BIS (1981).

Microbial analysis of dahi

Enumeration of lactoculture (LF-40) bacteria from T₀ sample of dahi.

Enumeration of viable lactobacilli count

The MRS agar having pH 6.4 with double layer was used for enumeration of viable lactobacilli counts of T₁, T₂ and T₃ samples of dahi.

Enumeration of coliforms

The zero and first dilutions of dahi samples were taken in duplicate into petri plates and then violet red bile agar (VRBA) 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 37°C for 24 hr and numbers of coliform colonies developed were counted as colony forming units (c.f.u.) per gram.

Enumeration of yeast and mould count (YMC)

The yeast and mould count (YMC) of the dahi samples was estimated as per the standard procedure given in IS : 1479 part-III (1962).

Organoleptic quality of dahi

Dahi samples prepared under different treatments were subjected to the organoleptic evaluation using judges. Each treatment was given code number, which was changed during each replication so as to avoid its identity. The scoring was recorded by using 9 point Hedonic scale.

Statistical analysis

The experimental data was analysed using completely randomized design (CRD) with four replication as described by Snedecor and Cochran(1994). The CRD modes used for analysis was

\[ Y_{ij} = \mu + \alpha_i + e_{ij} \]

Model assumption : \( \sum \alpha_i = 0 \)
Eij ~ N (0, åei²)
Where
Yij = Response of the jth dahi receiving ith culture
μ = General treatment
ái = Effect due to ith culture (I = 0, 1, 2 and 3)
eij = Random error which is assumed to be independent and normally distributed with mean zero and åei² level of significance.

RESULTS AND DISCUSSION

Chemical composition of milk

The mean percentage of fat and protein content of milk used for experiments were 4.02 and 3.24 %, respectively.

Chemical composition of dahi

Total solids

It is revealed that (Table 1) the total solids content of dahi samples ranged from 12.00 to 13.50 per cent.

Fat: It is revealed that (Table 1) the fat content of dahi samples was in the range of 3.90 to 4.20 per cent and coincided with the results of Laxminarayan and Shankar (1980). Fat content in dahi was more or less similar to fat content in milk used for dahi preparation.

Protein: The protein content in dahi samples ranged from 3.12 to 3.41 per cent (Table 1). Protein content in dahi was more or less similar to protein content in milk used for dahi preparation. Protein content of dahi samples coincided with result of Laxminarayan and Iya (1952), Rangappa and Achaya (1974), Laxminarayan and Shankar (1980) and De (1985).

Titratable Acidity ( % L.A. ): From the data given in the Table 1, it is revealed that all treatments significantly ( P < 0.05) differ from each other. The dahi (T3) prepared using combination of probiotic cultures Lb. acidophilus (0.5%) and Lb. delbrueckii subsp. bulgaricus (0.5%) showed higher acidity (0.99% L.A.) than other dahi samples tried in this experimentation. Dahi (T2) prepared by using Lb. delbrueckii sub sp. bulgaricus (1%) had more acidity than T1 (0.75% L.A.) and control dahi (T0).

Acidity content in dahi samples are in agreement with the reports of De(1985), and Ragappa and Acharya (1974).

Lactobacilli count of dahi: It is suggested that for getting maximum therapeutic value, the fermented milk product should contain population of viable cells of probiotic culture more than 10⁶ c.f.u./ml at the time of consumption. Keeping this fact in view, the products were studied for viable counts. The total counts of lactobacilli of T1, T2 and T3 samples of dahi were enumerated and depicted in Table 2. It is seen that dahi (T3) prepared by using combination of Lb. acidophilus and Lb. delbrueckii showed higher viable count 77 x 10⁶ c.f.u./gm. Dahi (T2) prepared by using Lb delbrueckii sub sp. bulgaricus showed viable lactobacilli count (47 x 10⁶ c.f.u./gm) higher than that found in T1 dahi sample (30 x 10⁶ c.f.u./

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total solids</th>
<th>Fat (%)</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>12.75</td>
<td>4.08</td>
<td>3.22</td>
</tr>
<tr>
<td>T1</td>
<td>12.82</td>
<td>4.02</td>
<td>3.16</td>
</tr>
<tr>
<td>T2</td>
<td>12.81</td>
<td>3.98</td>
<td>3.27</td>
</tr>
<tr>
<td>T3</td>
<td>12.85</td>
<td>4.08</td>
<td>3.27</td>
</tr>
</tbody>
</table>

TABLE 1. Chemical composition of dahi
TABLE 2. Total Lactobacilli count of dahi

<table>
<thead>
<tr>
<th>Dahi Type</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>13 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>20 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>37 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>50 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>30 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>30 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>26 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>72 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>60 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>47 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>50 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>60 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>91 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>107 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>77 x 10&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

TABLE 3. Coliform count of dahi

<table>
<thead>
<tr>
<th>Dahi Type</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

TABLE 4. Yeast and Mould count of dahi

<table>
<thead>
<tr>
<th>Dahi Type</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;0&lt;/sub&gt;</td>
<td>20</td>
<td>35</td>
<td>27</td>
<td>18</td>
<td>25</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>16</td>
<td>23</td>
<td>18</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>20</td>
<td>17</td>
<td>14</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>15</td>
<td>14</td>
<td>10</td>
<td>21</td>
<td>15</td>
</tr>
</tbody>
</table>

This count was slightly higher than that reported by Sheikh et al., (1970) (1.7 x 10<sup>4</sup> to 3 x 10<sup>6</sup> c.f.u./gm) and Mohanan et al., (1984) (11 x 10<sup>6</sup> to 350 x 10<sup>6</sup> c.f.u./gm).

Coliform, Yeast and Mold count: The fresh dahi samples were microbiologically analysed for contaminants viz., coliforms and yeast and moulds.

The presence of coliforms in dairy product is suggestive of insanitary conditions or practices followed during production, processing and inadequate care taken during post processing. From the data given in Table 3 it is revealed that the counts in products were in the range of 0 c.f.u./g to 7 c.f.u./g. It was below the standard prescribed by IS: 9617 (1980). These counts were more or less similar to the counts observed by Mohanan et al., (1984) (0 to 30 c.f.u./gm) and Dave et al., (1991).

YMC: 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. The counts of YMC were enumerated and given in Table 4. It is seen that the YMC remained within the limit (maximum 100 c.f.u./gm) prescribed by IS:9617(1980).

Organoleptic quality of dahi

The panel of 5 semi trained judges given fresh dahi samples for evaluation. The scores given by the judges for individual attributes were computed.

Aroma: It is revealed that (Table 5) T<sub>3</sub> dahi prepared by using Lb. acidophilus and Lb delbraeckii sub sp. bulgaricus in combination was ranked first so far as aroma is concerned. The mean aroma score of T<sub>2</sub> dahi sample prepared by using Lb. delbrieckii sub sp. bulgaricus was significantly lower (6.3) than the other dahi types tried in this investigation. It had off, unpleasant aroma. The T<sub>3</sub> dahi secured maximum aroma score (7.9).
However, it was at par with control T0 (7.75) and T1 dahi samples (7.63).

**Body and Texture:** Mean sensory score for body and texture of T3 and T0 dahi was the same but highest (7.98) and it was at par with T1 dahi (Table 5). The body and texture of T3 and T0 dahi was smooth and glossy while the cut surface was firm and free from crack and gas bubbles. T2 dahi sample received significantly lower score (6.13) than other dahi types prepared in this investigation.

**Colour and appearance:** Data recorded in Table 5 indicates score for colour and appearance of dahi under different treatments. The T3 dahi and control T0 dahi i.e. lactic culture (LF-40) obtained highest score (8.05 each) and was at par with T1 dahi sample (7.90) for colour and appearance. Control T0 and T3 dahi samples were attractive, and had uniform body with smooth, yellowish, glossy appearance without any free wheying off on surface of the product. T2 dahi secured significantly lower score (6.65) than rest of dahi types for colour and appearance as free wheying off was observed on the surface of the product.

**Taste**

The sensory score values obtained for taste of different experimental samples of dahi are presented in Table 5. It is revealed that the T3 dahi secured highest score (7.97) than other samples and was at par with T1 and control dahi (T0) sample. Treatment T3 ranked first so far taste is concerned as it contained delicate and clean acid taste. The T2 dahi secured significantly lower score (5.87) than rest of the dahi samples because of it was lacking clean acid taste.

**Conclusion**

It could be concluded that the delicious dahi with pleasant aroma can be prepared by using probiotic cultures *viz.*, lactobacillus acidophilus and lactobacillus delbrueckii subsp bulgaricus alone or in combination containing viable lactobacilli counts more than 10<sup>6</sup>c.f.u./gm.

**REFERENCES**


* The treatments mean having same superscripts are statistically non-significant.


