Comparative analysis of the effect of herbs on diverse microbiological properties of yoghurt at different storage periods

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Abstract
Yoghurt is rich in nutrients like potassium, calcium, protein and vitamin B and has beneficial effects on human health by supplying prebiotic and probiotic bacteria. It helps to strengthen the immune system, improves lactose digestion and gastrointestinal conditions including lactose intolerance, constipation, diarrhoea, colon cancer, inflammatory bowel disease and allergies. Six batches of different herbal probiotic yoghurt were prepared using fresh milk by inoculated with 4 per cent of yoghurt cultures containing Lactobacillus delbrueckii subsp. bulgaricus, and Streptococcus salivarius subsp. thermophilus. In the viability study of probiotics organisms, it was revealed that there was significant (P<0.05) reduction in the viability of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (log10cfu/ml) were noticed in all the herbal yoghurt including plain yoghurt during storage period of 21 days and wherein the reduction was high in AGY, CIY and HSY compared with PY and ABY and this may be attributed to slight acidic nature of herb. However the count of probiotic strains was maintained at 7 log units for all herb treated yoghurts throughout storage period of 21 days.

Keywords: Yoghurt, probiotic, Streptococcus salivarius, Lactobacillus delbrueckii, microbial growth

Introduction
Dairy products, particularly those containing prebiotics, probiotics and symbiotics are most popular in this category of foods. Probiosis can be defined as ‘the positive effect of consumption of fermented dairy products with the culture of lactic acid bacteria (LAB) on the equilibrium maintainance of intestinal microflora’ (Tomasik and Tomasik, 2003) [11]. Yoghurt is rich in nutrients like potassium, calcium, protein and vitamin B and has beneficial effects on human health by supplying prebiotic and probiotic bacteria. It helps to strengthen the immune system, improves lactose digestion and gastrointestinal conditions including lactose intolerance, constipation, diarrhoea, colon cancer, inflammatory bowel disease and allergies (Fitzgerald et al., 2004) [2]. Apart from this, yoghurt has also been associated with a number of health benefits including anti-carcinogenic properties, anti-mutagenic properties, reduction in serum cholesterol and management of hypertension, immune system stimulation and suppression of Helicobacter pylori infection (Shah, 2000b) [10]. The beneficial health effects of yoghurt have been partly linked to the proteolysis products produced during fermentation and storage. During fermentation, LAB produces a range of secondary metabolites, some of which have been associated with health promoting properties of which the notable ones are the B-vitamins and bioactive peptides.

The present study investigate infusion of the commonly used herbs Aloe barbadensis (Aloe vera), Anethum graveolence (dill), Costus igneus (insulin plant) and Hibiscus sabdariffa (roselle) on yoghurt formation and also to evaluate the Effect of herbs on microbiological properties of yoghurt at different storage periods.

Materials and Methods
Fresh cow milk obtained from the Department of ILFC, Veterinary College and Research Institute, Namakkal – 637 002 was used. Skim milk powder testing 5 and 95 per cent moisture and solubility, respectively was purchased locally (AAVIN). Commercially available good quality cane sugar was used in the current study. Freeze dried DVS cultures containing yoghurt bacteria Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus salivarius ssp.
thermophilus obtained from Chr. Hansen, Denmark, were used in this study. Herbal infusions like Aloe barbadensis collected from campus of Veterinary College and Research Institute, Namakkal. Anethum graveolens purchased from local markets of Kolar district, Karnataka. Costus igneus and Hibiscus Sabdariffa collected from Horticulture College, GKVK campus, Bangalore was employed in the study.

Preparation of probiotic yoghurt
Six batches of different herbal probiotic yoghurt were prepared using fresh milk. Skim milk powder at the rate of 2 per cent (w/v) and sugar at the rate of 6 per cent (w/v) were added to it and homogenized at 1000 psi. The contents were mixed well and pasteurized at 85 °C for 30 minutes, cooled to room temperature and inoculated with 4 per cent of yoghurt cultures containing Lactobacillus delbrueckii subsp. bulgaricus, and Streptococcus salivarius subsp. thermophilus. Different concentrations of herbs were added before incubation and mixed well and incubated at 42 °C for 4 to 5 hours and stored at 4 °C when the pH was reduced to 4.5. These yoghurts were then placed in the refrigerator for up to 21 days.

Herbal yoghurt
Five different concentrations of herbal yoghurt were prepared from each different herb for standardization and were denoted as
1. PY = Plain yoghurt without adding any herb as a standard yoghurt
2. ABY = Aloe barbadensis incorporated yoghurt
3. AGY = Anethum graveolens incorporated yoghurt
4. CIY = Costus igneus incorporated yoghurt
5. HSY = Hibiscus Sabdariffa incorporated yoghurt

Thus prepared herbal yoghurt was subjected to evaluate the microbiological properties of yoghurt in detail at different storage periods.

Storage period used in the current study
Herbal yoghurt was subjected to evaluate microbiological properties of yoghurt in detail at different storage periods like zero, 7th, 14th and 21st day.

Results
The outcome of the current study is presented and depicted as tables 1-4 and figure 1-2.

Viability of Streptococcus salivarius subsp. thermophilus (log10 cfu/ml) in herbal yoghurt during storage period
Effect of herbs on viability of Streptococcus salivarius subsp. thermophilus (mean±SE) in herbal yoghurt during storage period of zero day, 7th day, 14th day and 21st day were represented as Tables 1, 2 and figure 1.

Table 1: Viability of probiotic Streptococcus salivarius subsp. thermophilus (log10 cfu/ml) in herbal yoghurt during storage period (mean±SE)

<table>
<thead>
<tr>
<th>Days</th>
<th>PY</th>
<th>ABY</th>
<th>AGY</th>
<th>CIY</th>
<th>HSY</th>
<th>Storage mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>zero day</td>
<td>8.93±0.01</td>
<td>8.85±0.01</td>
<td>8.81±0.01</td>
<td>8.84±0.01</td>
<td>8.82±0.01</td>
<td>8.84±0.002</td>
</tr>
<tr>
<td>7th day</td>
<td>8.95±0.01</td>
<td>8.90±0.01</td>
<td>8.83±0.01</td>
<td>8.84±0.01</td>
<td>8.84±0.01</td>
<td>8.87±0.002</td>
</tr>
<tr>
<td>14th day</td>
<td>8.81±0.01</td>
<td>8.76±0.01</td>
<td>8.43±0.03</td>
<td>8.40±0.03</td>
<td>8.60±0.01</td>
<td>8.6S±0.01</td>
</tr>
<tr>
<td>21st day</td>
<td>8.14±0.03</td>
<td>7.75±0.06</td>
<td>7.87±0.02</td>
<td>7.86±0.02</td>
<td>7.50±0.06</td>
<td>7.82±0.01</td>
</tr>
<tr>
<td>Treatment mean±SE</td>
<td>8.71±0.01</td>
<td>8.56±0.02</td>
<td>8.48±0.02</td>
<td>8.47±0.01</td>
<td>8.44±0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Means bearing same superscript column wise and row wise was not significant.

Table 2: ANOVA (Multivariate) of viability of probiotic Streptococcus salivarius subsp. thermophilus in herbal yoghurt during storage period

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MSS</th>
<th>F-value</th>
<th>P-value</th>
<th>Error</th>
<th>df</th>
<th>MSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>0.282</td>
<td>13.541</td>
<td>0.00</td>
<td>100</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td>3</td>
<td>7.209</td>
<td>345.669</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage*Treatment</td>
<td>12</td>
<td>0.097</td>
<td>4.677</td>
<td>0.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reduction in the viability of Streptococcus salivarius subsp. thermophilus (log10 cfu/ml) was noticed in all the treated herbal yoghurts including plain yoghurt. On zero day, the count for PY was 8.93±0.01 followed by ABY, HSY, CIY and AGY of 8.85±0.01, 8.82±0.01, 8.81±0.01 and 8.81±0.01, respectively which got reduced to 8.14±0.03, 7.75±0.06, 7.87±0.02, 7.86±0.02 and 7.50±0.06 for PY, ABY, AGY, CIY and HSY, respectively on 21st day.

Fig 1: Viability of probiotic Streptococcus salivarius subsp. thermophilus (log10 cfu/ml) in herbal yoghurt during storage period
From the statistical analysis of the table 1, it was observed that there was a significant ($P<0.05$) reduction in the storage mean±SE value of viability from 8.84±0.002 to 7.82±0.01 during storage from day zero to 21, respectively. Overall treatments the count for PY and ABY were significantly ($P<0.05$) higher (8.71±0.01 and 8.56±0.02, respectively) on comparison with AGY, CIY and HSY with the values of 8.33±0.003 and 7.00±0.01, respectively.

### Table 3: Viability of probiotic lactobacillus delbrueckii subsp. bulgaricus (log10 cfu/ml) in herbal yoghurt during storage period (mean±SE)

<table>
<thead>
<tr>
<th>Days</th>
<th>PY</th>
<th>ABY</th>
<th>AGY</th>
<th>CIY</th>
<th>HSY</th>
<th>Storage mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>zero day</td>
<td>8.31±0.01</td>
<td>8.31±0.02</td>
<td>8.36±0.02</td>
<td>8.34±0.02</td>
<td>8.31±0.01</td>
<td>8.33±0.003</td>
</tr>
<tr>
<td>7th day</td>
<td>8.35±0.01</td>
<td>8.47±0.01</td>
<td>8.43±0.02</td>
<td>8.37±0.03</td>
<td>8.35±0.01</td>
<td>8.39±0.004</td>
</tr>
<tr>
<td>14th day</td>
<td>8.14±0.02</td>
<td>8.39±0.01</td>
<td>7.96±0.02</td>
<td>7.95±0.02</td>
<td>8.14±0.02</td>
<td>8.14±0.01</td>
</tr>
<tr>
<td>21st day</td>
<td>7.07±0.05</td>
<td>7.22±0.05</td>
<td>6.95±0.07</td>
<td>6.69±0.05</td>
<td>7.07±0.07</td>
<td>7.00±0.01</td>
</tr>
<tr>
<td>Treatment mean±SE</td>
<td>7.96±0.02</td>
<td>8.10±0.02</td>
<td>7.92±0.03</td>
<td>7.84±0.03</td>
<td>7.96±0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

abc means bearing same superscript column wise and row wise was not significant.

### Table 4: ANOVA (Multivariate) of viability of probiotic lactobacillus delbrueckii subsp. bulgaricus in herbal yoghurt during storage period

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MSS</th>
<th>F-value</th>
<th>P-value</th>
<th>Error df</th>
<th>Error MSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>4</td>
<td>0.207</td>
<td>4.714</td>
<td>0.00</td>
<td>100</td>
<td>0.044</td>
</tr>
<tr>
<td>Storage</td>
<td>3</td>
<td>12.685</td>
<td>288.780</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage*Treatment</td>
<td>12</td>
<td>0.097</td>
<td>4.677</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The present study is in agreement with previous studies of Lawin and Kongbangkerd (2010) [5] that the survival of L. bulgaricus, S. thermophilus and L. casei decreased throughout the storage period of 21 days on addition of different levels of roselle (Hibiscus sabdariffa) syrup with probiotic count range of 8-9 log cfu/g and ensured that the yoghurt could be claimed for human health. Panesar and Shinde (2011) [6] revealed that the addition of Aloe vera juice blend in to yoghurt decreases Lactobacillus acidophilus count from 39.7×10^9 cfu/ml to 32.1×10^9 cfu/ml and Bifidobacterium bifidum count from 16.9×10^9 cfu/ml to 7.3×10^9 cfu/ml during the storage period of 28 days. Behrad et al. (2012) [1] noticed that addition of licorice resulted in significantly higher count of yoghurt cultures compared to plain yoghurt during the storage period of 28 days. Marhamatizadeh et al. (2012) [6] determined that the shelf life of Anethum graveolens incorporated yoghurts was 21 days during which the bacterial count decreased but not less than 10^9 and it was observed in the study that count of Lactobacillus acidophilus and Bifidobacterium bifidum was 6.75×10^9 and

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Discussion

There was significant ($P<0.05$) reduction in the viability of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (log$_{10}$ cfu/ml) were noticed in all the herbal yoghurt including plain yoghurt with the values 8.84±0.002 and 7.82±0.01 for S.thermophilus and 8.33±0.003 and 7.00±0.01 for L.bulgaricus on zero day to 21st day, respectively (Table 1).
10.75x10^{10} on first day and 10x10^{10} and 9.5x10^{10} in 15th day, respectively on addition of 0.6% per cent Anethum graveolens.

Marhamatizadeh et al. (2013) [7] reported effect of green tea extract (0.9 per cent) on Lactobacillus acidophilus and olive leaf extract (0.9 per cent) on Bifidobacterium bifidum in yoghurt during storage and recorded Lactobacillus acidophilus count of 23.5x10^{10} cfu/ml on first day and 23.5x10^{10} cfu/ml on 21st day and Bifidobacterium bifidum count of 19.25x10^{10} cfu/ml on first day and 45.75x10^{10} cfu/ml on 21st day.

Houshang et al. (2014) [4] concluded that increasing the level of addition of Aloe vera has a positive influence on the growth and viability of Lactobacillus acidophilus and Bifidobacterium bifidum in probiotic milk and yoghurts. The present study concluded that the addition of extracts of Aloe barbadensis, Anethum graveolens, Costus igneus and Hibiscus sabdariffa did not affect the growth and viability of the probiotic organism in the yoghurt during the storage period of 21 days and all the herbal yoghurts have been found to have the count above 6 log<sub>10</sub>

In order to obtain the desired health effects, probiotic organisms should be present in a food at a minimum concentration of 10<sup>-4</sup>-10<sup>8</sup> cfu/g (Gomes and Malcata, 1999) [3]. Such high numbers have been recommended to compensate for possible losses in the numbers of the probiotic organisms during passage through the stomach and intestine. Therefore, in order to provide the therapeutic benefits, attempts to increase viability in dairy products have drawn the attention of researchers in recent years. On the other hand, there is much evidence to show that S. thermophilus and L. bulgaricus survive at high concentration (>10<sup>7</sup> cfu/g) in yoghurts after manufacture until the time of consumption (Rohm et al., 1990) [9].

Conclusion

In the viability study of probiotics organisms, it was revealed that there was significant (P<0.05) reduction in the viability of Streptococcus salivarius subsp. thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (log10 cfu/ml) were noticed in all the herbal yoghurt including plain yoghurt during storage period of 21 days and wherein the reduction was high in AGY, CIY and HSY compared with PY and ABY and this may be attributed to slight acidic nature of herb. But, the count of probiotic strains was maintained at 7 log units for all herb treated yoghurts throughout storage period of 21 days.

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References