ABSTRACT
Eating dairy products, such as yoghurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations. Probiotics are increasingly finding use as dietary supplements in processed foods and are known for improving the host intestinal microbial balance and several potential health benefits. The use of Aloe vera juice in the probiotic foods can be a promising trend towards use of herb as well as functional ingredients in the dairy foods. In the present investigation, Aloe vera fortified probiotic yoghurt was prepared by the combination of Aloe vera juice, skim milk powder and two probiotic cultures viz., Lactobacillus acidophilus and Bifidobacterium bifidum. Physical and chemical evaluation of Aloe vera juice and Aloe vera fortified yoghurt were performed and the effect of storage on syneresis, pH, acidity, sugars, proteins of Aloe vera fortified probiotic yoghurt was assessed for storage study. Syneresis was increased from 4.6 to 7.9 % (v/w), pH decreased from 4.8 to 2.7 and 4.8 to 2.3, acidity increased from 0.31 to 0.92% and 0.31 to 0.96%, sugar concentration decreased 3.71 to 2.74 and 3.71 to 2.58 μg/ml and proteins increased from 4.2 to 4.58 and 4.2 to 4.53% for L. acidophilus and B. bifidum respectively. Yoghurt prepared with two different cultures L. acidophilus and B. bifidum resulted in minor differences. Potentially important changes were observed in the different yoghurt samples within 28 day storage period.

Keywords: Yoghurt, Aloe vera, Probiotic, Physico-chemical property.

INTRODUCTION
The fermented milk product such as cheeses and yoghurts into the diet of human being is thought to date back to the dawn of the civilization 1. The use of yoghurt as a calcium source has made it one of the most rapidly growing dairy products, but presently it is more than just a calcium source. Yoghurt, Kefir and similar fermented milk products are on the way to becoming major nutraceuticals aimed at treating a variety of disease conditions 2. Special types of yoghurt manufactured for dietetic and/or therapeutic purposes are known as bio-yoghurt 3. The concept of probiotics evolved from a theory first proposed by Nobel Prize winning Russian scientist, Elie Metchnikoff who suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products. Probiotic bacteria can be found worldwide in a variety of products, including conventional food products, dietary supplements and medicinal foods 4. Yoghurt is a unique dairy product because the starter cultures actually produce that β-galactosidase enzyme which breaks the main carbohydrate in milk during fermentation. Thus, the milk sugar in yoghurt is more easily digested, even for lactose-intolerant individuals 5. For about hundred years, Liliaceae family has been used as functional agent in different foods and also has been applied as a traditional medicine for treatment of digestive and viral diseases. Aloe vera is a member of the lily family (Liliaceae) and use of Aloe vera juice in the probiotic yoghurt will be a promising trend towards use of herb as well as functional ingredients in the dairy foods. The addition of Aloe vera juice may improve the activity of starter cultures in the product and may account for better textural properties leading to varying percentages of syneresis by reducing the amount of liquid separation in gels and owing to
further coagulation of the low-fat set probiotic yoghurt samples. Probiotics are gaining enormous attention because of their established health effects such as anti-diarrheal, anti-pathogenic, anti-diabetic, anti-cholesterol and anti-cancer activities etc. 6,8. Yoghurts are also extremely healthy having high content of antioxidants, vitamins, minerals, dietary fibers and many other beneficial nutrients; hence could serve as a good medium for cultivating probiotics 9,10. Therefore, in present investigation, an attempt was made to prepare low-fat set Aloe vera fortified yoghurt by using two different probiotics viz., Lactobacillus acidophilus and Bifidobacterium bifidum and analyze the survival of sample by physico-chemical analysis.

MATERIALS AND METHODS

Preparation of Aloe vera Juice
Freshly harvested, matured Aloe vera leaves were cut from the bottom of the plant with a sharp knife, and washed thoroughly. Then, using a sharp knife, the rough edges were removed. The slimy mucilage and transparent gel was scooped out with a spoon from the Aloe vera leaf. It was made sure that the yellow sap exuded from the green part of the leaf was discarded properly. This is called aloin that causes irritation reactions. Aloe vera gel was then, macerated in a grinder, was subsequently filtered to separate the fiber and get the juice used for production. It was preserved in the refrigerator for further use.

Preparation of Low-fat Set Aloe vera Fortified Yoghurt
The reconstituted skim milk for low-fat Aloe vera fortified set yoghurt was prepared by using 16.57gm skim milk powder in Aloe vera juice and water blend, 25 and 75ml, respectively. The prepared reconstituted skim milk was heated and pasteurized properly at 82-85°C for 12-15 min. The reconstituted skim milk was cooled to 45°C. The inoculation was done with starter cultures and two different probiotics L. acidophilus and B. bifidum separately and kept for incubation at 37°C for 8 hrs. After incubation the samples were kept under refrigerated condition at 4°C. Physical and chemical evaluation of Aloe vera juice and Aloe vera fortified yoghurt were performed 11.

Analysis of Physico-Chemical Characteristics

Leaf Fillet and Juice Yield
Various leaf parameters were recorded for Aloe vera. Harvested leaves were manually filleted in the laboratory. Whole leaves were washed and scrubbed to remove mud and bitter exudates on the rind surface, after washing process, leaves were weighed. The sides, base and tip of the leaves were then removed, and the inner leaf portion cut longitudinally into strips. The gel parenchyma was then cut away from the rind with a sharp knife. Gel fillet weights were recorded for each leaf. Gel fillets were then liquidized by mixer and filtered through Whatman filter paper applying vacuum until all liquid was removed. Juice recovered was weighed.

Percent Gel Fillet Yield and Percent Juice Yield
Percent gel fillet and juice yield were measured by following formula 12.

\[
\text{% Gel fillet yield} = \frac{\text{Weight of gel fillet}}{\text{Weight of leaf}} \times 100
\]

\[
\text{% Juice yield} = \frac{\text{Weight of juice}}{\text{Weight of gel fillet}} \times 100
\]
Chemical Composition of Aloe vera Fortified Yoghurt

Moisture Content
The estimation of moisture content was carried out \(^{13}\). The samples were weighed in a clean, dry petri plates and placed in a hot air oven at 100°C for 2 hrs. It was then removed from the oven and cooled in a desiccator and weighed. The Petri plates were then again kept in the oven and repeated the steps of heating, cooling and weighing after an interval of 30 min until the difference between the successive weights was less than 0.1 gm.

**Calculation:** The % total solids were calculated by using the formula:

\[
\text{% Total solids} = \frac{\text{Dried sample weight}}{\text{Fresh sample weight}} \times 100
\]

Moisture content of the Aloe vera juice was calculated as:

% Moisture content = 100 - % Total solids

Total Soluble Solids
The estimation of total soluble solids was done \(^{13}\). The total soluble solids (TSS) of Aloe vera juice were measured by using hand refractometer. The refractometer reading was determined by placing a drop of gel on the prism and reading the corresponding percentage from direct reading. TSS was expressed as °brix at room temperature i.e. 24°C.

Total Ash
The estimation of total ash was carried out by AOAC method \(^{14}\). A sample was weighed (5 gm) into ashing dish which was ignited, cooled in desiccator, and weighed soon after reaching room temperature. Ignited in furnace at 550°C until light gray ash results and constant weight is obtained. Percent ash content was calculated by knowing the difference between the initial and final weight.

Determination of Syneresis
Susceptibility of yoghurt to syneresis was determined by centrifuging 20gm of sample at 500 rpm for 5 min and weighing the supernatant \(^{15}\). Measuring the amount of supernatent recovered (%v/w). Percent syneresis calculated as:

\[
\text{% syneresis} = \frac{\text{Vol.of supernatent}}{\text{Weight of sample}} \times 100
\]

pH
The pH electrode was dipped in the yoghurt sample and pH of the sample was noted from the display.

Acidity
10ml of sample was taken with pipette in a 100ml beaker and added 4-5 drops of phenolphthalein indicator. Then titrated it against 0.1% NaOH till a persistent pink colour was obtained.

**Calculation**

\[
\text{Titrate acidity} = \frac{\text{Volume of NaOH used} \times \text{Normality}}{\text{Volume of sample taken}} \times 9
\]

DNS Method to Test Reducing Sugars
Estimation of reducing sugars was done by dinitrosalicylic acid (DNS) method which is used to detect the presence of free carbonyl group (C=O) \(^{16}\).

Protein Estimation
Estimation of protein was done by following Folin-Lowry method \(^{17}\).

RESULTS AND DISCUSSION
The purpose of determination of the physical and chemical properties of Aloe vera leaves were to get idea about the nutritional quality of ingredients and its suitability to prepare good quality of final product. Gel fillet weight in the present study was found to be 232.6-374.4 gm per leaf (Table 1). Using the hand filleting technique, the average yield of gel fillet per leaf was approximately 44.06 % (w/w). The results are in accordance with the reported literature where average gel fillet yield was found to be more than 42 % (w/w) \(^{18}\). Juice weight ranges between 162.2-268.6 gm per leaf. When considering gel liquid yields, the overall recovery of juice from gel fillet (w/w) was up to 69.55 % (w/w). This was mainly influenced by the water content of the gel.

Physico-Chemical Analysis of Aloe vera Juice
Various physico-chemical attributes of Aloe vera juice obtained from the leaf are shown in Table 2. Moisture content was found to be 96.4%. Total Solids (TS) of Aloe vera juice (by oven drying) and total soluble solid (refractometer) was found to be 1.80 % (w/w) and 1.8°brix respectively. In earlier studies the total solids in aloe gel was found to be 1.5% (w/w) \(^{19}\). The ash content was relatively high in the gel fraction where it accounted for up to 21.4 % (w/w) of the dry matter. Aloe vera pH was found to be 4.3 and previous studies also find the same pH of Aloe vera juice \(^{18}\). Acidity was found to be 0.28%, sugar and protein content to be 4.12µg/ml and 5.38µg/ml respectively.

Physico-Chemical Analysis of Aloe vera Fortified Yoghurt
The moisture content of the Aloe vera yoghurt was 81.28% which was calculated on wet basis. Total
solids by oven drying were 13.5% and total soluble solids (Refractometer) were 13° Brix. Ash content was 11.6%, pH and acidity of the yoghurt was 4.8 and 0.34% respectively. The sugar and protein content of the Aloe vera yoghurt was found to be 3.52µg/ml 4.68 µg/ml respectively (Table 3).

Effect of Storage on Syneresis
When yoghurt was inoculated with cultures, an increase in the syneresis was observed in all the cultures with storage time. Syneresis was observed at regular intervals of 7 days up to 28 days. The initial syneresis of fresh yoghurt was found to be 4.6% and 4.1% (v/w) for L. acidophilus and B. bifidum and after storage period of 28 days values reached upto 7.9% and 7.4% (v/w) for L. acidophilus and B. bifidum respectively. Rapid increase in syneresis was observed after 14 days (Fig. 1). During the period of storage the value of syneresis increased as time period increases. L. acidophilus yoghurt showed more syneresis than B. bifidum yoghurt. Previous studies also reported that syneresis increases with the passage of time 11 due to increase of acidity and decreases of pH 20.

Effect of Storage on pH
During storage, the pH in fresh samples was decreased for L. acidophilus yoghurt and B. bifidum yoghurt from 4.8 to 2.7 and 4.8 to 2.3 respectively (Fig. 2). Thus there was a decrease in pH constantly throughout the storage period. There is continuously decrease in pH throughout storage period11 and decrease may be due to microorganism activity, post acidification during storage or residual enzymes produced by starters during fermentation 21-23.

Effect of Storage on Acidity
The decrease in pH was concurrent with the increase in acidity of yoghurt samples of L. acidophilus and B. bifidum. Acidity was checked at initial day and it was 0.31% for both the yoghurt and continuously increased up to 28th day and reported to be 0.92% and 0.96% for L. acidophilus and B. bifidum yoghurt respectively (Fig. 3). The faster increase in acidity is expressed due to its lower buffering capacity and high content of non protein nitrogen and vitamins which are needed for fast growing micro-organisms 24-25.

Effect of Storage on Sugar Concentration
The initial concentration of sugar in yoghurt sample inoculated with L. acidophilus and B. bifidum was 3.71µg/ml which increased continuously from 7 days up to 14 days i.e. 3.96 and 3.88 for L.acidophilus and B.bifidum. After this period conc. of sugar decreased from 21 to 28 days. On the 28th day conc. of sugar was found to be 2.74 µg/ml and 2.68 µg/ml for L. acidophilus and B. bifidum respectively (Fig. 4).

Effect of Storage on Protein Content
The protein content was also checked on regular intervals of 7 days up to 28 days. Initial protein content was 4.20 and 4.16µg/ml for L. acidophilus and B. bifidum as the storage period starts for both the yoghurt samples which showed decrease in values upto 4.1 µg/ml and 4.13 µg/ml after 7 days for L. acidophilus and B. bifidum yoghurt. After that it increases continuously during the whole storage period and reached upto 4.58 µg/ml and 4.53 µg/ml for L.acidophilus and B. bifidum yoghurt (Fig. 5). The increase in protein content in yoghurt depends on the proteolytic activity of lactic acid bacteria, which hydrolyses proteins into peptides and amino acids 26.

CONCLUSION
Use of Aloe vera juice in the probiotic foods can be a promising trend towards use of herb as functional ingredients in the dairy food. Aloe vera fortified yoghurt was prepared by using two different probiotics L. acidophilus and B. bifidum and to study the physico-chemical characteristics of Aloe vera fortified probiotic yoghurt. Since in present study, all the cultures were found to be able to survive in the yoghurt with high acidity and low pH therefore, it could be advocated that yoghurt could be exploited as carrier/medium for the fermentation and delivery of probiotics, and these probiotic fortified yoghurt products could be used as a functional healthy beverage for the better health and nutrition of people.
Table 2

Physico-Chemical Properties of Aloe vera Juice

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Moisture content</td>
<td>96.4 %</td>
</tr>
<tr>
<td>2.</td>
<td>Total solids (oven drying)</td>
<td>1.80 %</td>
</tr>
<tr>
<td>3.</td>
<td>Total soluble solids (Refractometer)</td>
<td>1.8˚ Brix</td>
</tr>
<tr>
<td>4.</td>
<td>Total ash</td>
<td>21.4%</td>
</tr>
<tr>
<td>5.</td>
<td>pH</td>
<td>4.3</td>
</tr>
<tr>
<td>6.</td>
<td>Acidity</td>
<td>0.28%</td>
</tr>
<tr>
<td>7.</td>
<td>Sugars</td>
<td>4.12µg/ml</td>
</tr>
<tr>
<td>8.</td>
<td>Protein Content(%, w/w)</td>
<td>5.38 µg/ml</td>
</tr>
</tbody>
</table>
Fig 2
Effect of Storage on pH of Aloe vera Fortified Yoghurt

Fig 3
Effect of Storage on Acidity of Aloe vera Fortified Yoghurt

Fig 4
Effect of Storage on Sugar Conc. of Aloe vera Fortified Yoghurt
Fig 5
Effect of Storage on Protein Content of Aloe vera Fortified Yoghurt

REFERENCES


