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Research Article

Physico-Chemical Analysis of *Aloe vera* Fortified Probiotic Yoghurt

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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% and 4.1 to 7.4% (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

Starter Cultures

The yoghurt culture used for preparation of *Aloe vera* fortified yoghurt, containing *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (obtained from market available product yakult) and two probiotic cultures viz., *Lactobacillus acidophilus* (MTCC No. 10307) was obtained from IMTECH, Chandigarh and *Bifidobacterium bifidum* (NCDC No. 0235) was obtained from NDRI Karnal.

Media for Culture Activation

S. thermophilus and *L. acidophilus* were activated in litmus milk. The *L. bulgaricus* was activated using 10 % (w/v) skim milk and *B. bifidum* were activated in MRS containing 0.05 % (w/v) cysteine and pyruvate.

Aloe vera

The fresh *Aloe vera* was obtained from nursery of Patiala, Punjab, India.

Activation and Maintenance of Cultures

The cultures *L. acidophilus* and *B. bifidum*, obtained in the form of vacuum sealed ampoules were break opened in aseptic conditions and added 4-5 drops of sterilized skim milk or MRS broth with the help of sterilized pipette. The contents were mixed gently and then transferred to test tubes containing 10-20 ml sterilized skim milk or MRS broth. The test tubes were incubated at 37 ± 1ºC. Further sub-culturing was carried out by transferring them into sterilized skim milk or MRS broth at 1% (v/v). These fully activated cultures were stored in the refrigerator. The cultures were maintained by sub culturing, aseptically at fortnight intervals and stored in refrigerator, until further use. A 24-hour-old bulk culture was used for the preparation of low-fat set *Aloe vera* fortified probiotic yoghurt.

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

Physical Properties of *Aloe vera*

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

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 tatal 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. Synersis was observed at regular intervals of 7 days upto 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 synersis 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 synersis 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 decrease in pH constantly throughout the storage period. There is continuously decrease in pH throughout storage period[11] 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 upto 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 upto 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 upto 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* respectively (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 vear* 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 1
Physical Analysis of *Aloe vera* Leaf

Leaf No.	Leaf Weight (gm)	Gel Fillet Weight (gm)	Gel Fillet Yield (%)	Juice Weight (gm)	Juice Yield (%)
1.	669.1	374.4	55.32	268.6	70.8
2.	626.8	232.6	35.18	162.2	68.20
3.	629.3	252.5	41.68	174.9	69.64

Table 2
Physico-Chemical Properties of *Aloe vera* Juice

S. No.	Parameters	Quantity
1.	Moisture content	96.4 %
2.	Total solids (oven drying)	1.80 %
3.	Total soluble solids (Refractometer)	1.8° Brix
4.	Total ash	21.4%
5.	pH	4.3
6.	Acidity	0.28%
7.	Sugars	4.12µg/ml
8.	Protein Content(%, w/w)	5.38 µg/ml

Table 3
Physico-Chemical Analysis of *Aloe vera* Yoghurt

S. No.	Parameters	Quantity
1	Moisture content (on wet basis)	81.28 %
2	Total solids (oven drying)	13.5 %
3	Total soluble solids (refractometer)	13° Brix
4	Total ash content	11.6 %
5	pH	4.8
6	Acidity	0.34%
7	Sugars	3.52 µg/ml
8	Protein content(%, w/w)	4.68 µg/ml

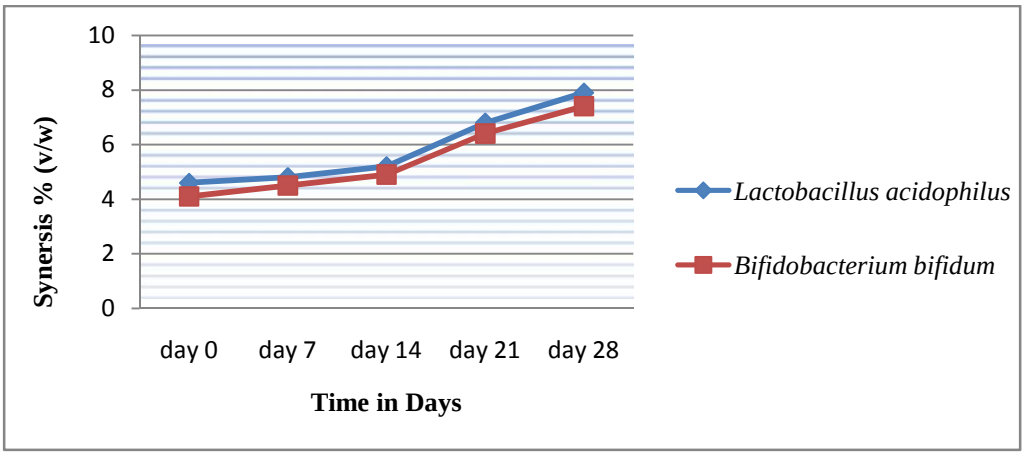

Fig 1
Effect of Storage on of *Aloe vera* Fortified Yoghurt

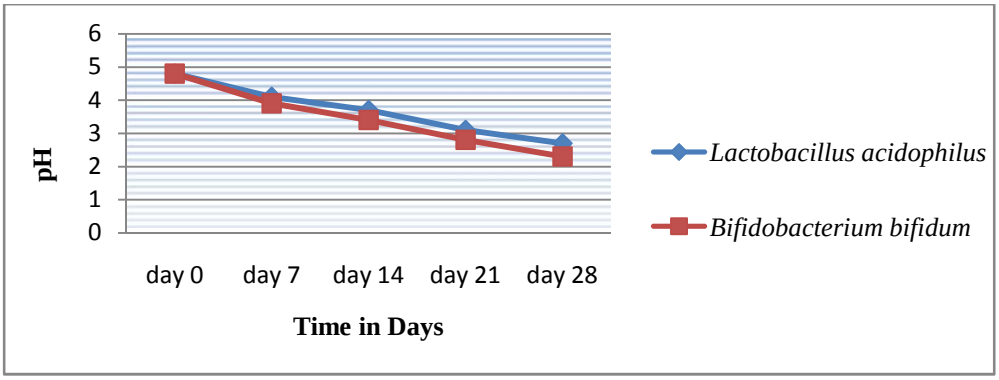

Fig 2
Effect of Storage on pH of *Aloe vera* Fortified Yoghurt

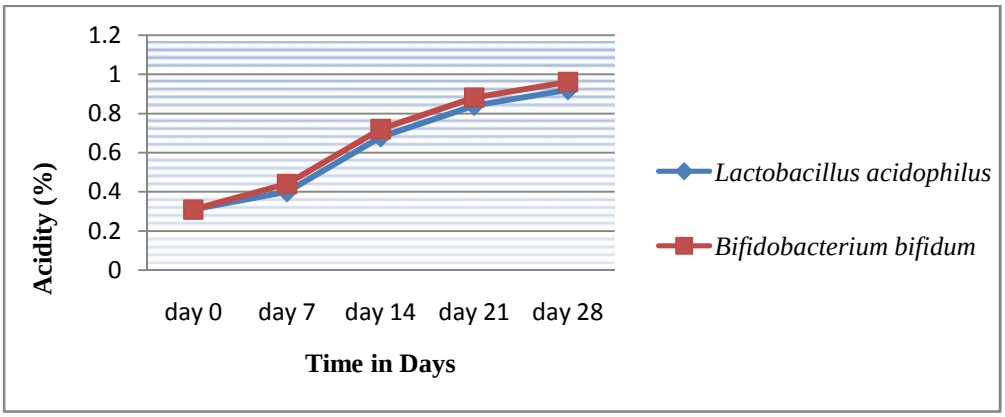

Fig 3
Effect of Storage on Acidity of *Aloe vera* Fortified Yoghurt

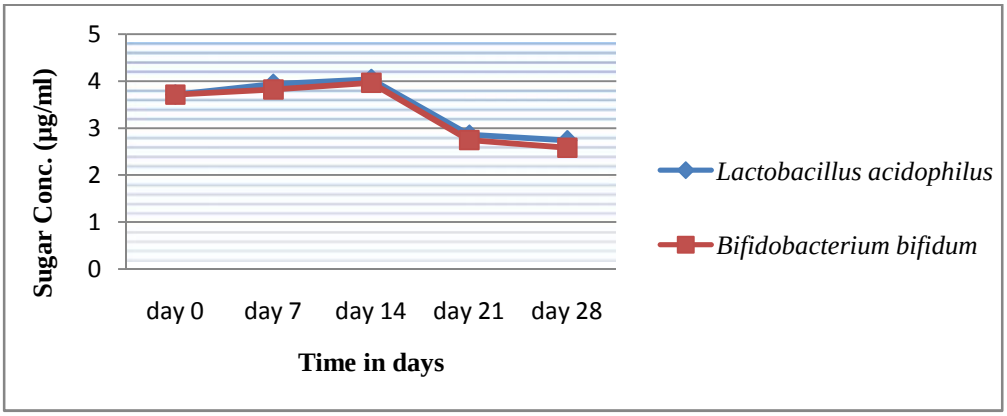

Fig 4
Effect of Storage on Sugar Conc. of *Aloe vera* Fortified Yoghurt

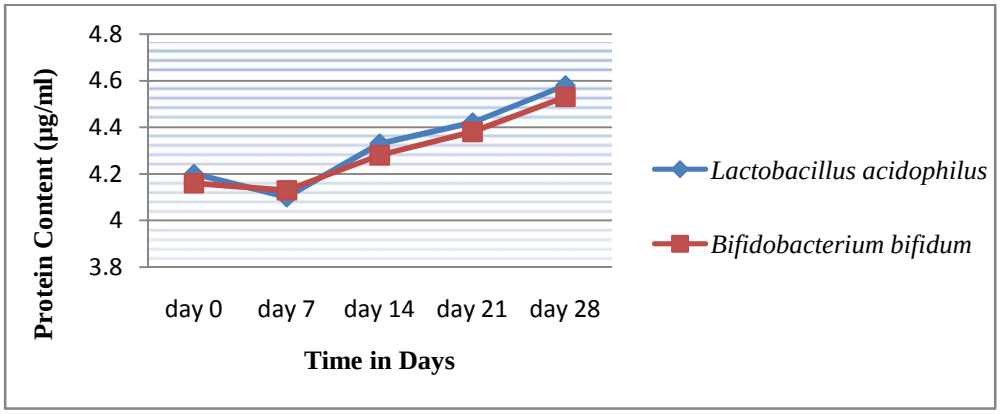

Fig 5
Effect of Storage on Protein Content of *Aloe vera* Fortified Yoghurt

REFERENCES

1. Mckinley MC. The Nutrition and Health Benefits of Yoghurt. Int. J. Dairy Technol. 2005; 58(1): 1-12.
2. Katz F. Active Cultures Add Functions to Yogurt and Other foods. Food Technol. 2001; 55: 46-49.
3. Tamime AY, Robinson RK. Yoghurt: Science and Technology, 2000; 2nd Ed., 19: 375-376.
4. Fioramonti J, Theodorou V, Bureno L. Probiotics: what are they. What are their effects on gut physiology. Best Pract. Res. Clin. Gastroenterl. 2003; 17(5): 711-724.
5. Fernandes CF, Shahani KM. Anticarcinogenic and Immunological Properties of Dietary *Lactobacilli.* J. Food Protect. 1990; 53(8): 704–10.
6. Roberfroid MB. Concepts and strategy of functional food science; the eurpeaon perspective. Ain. J. Clin. Nutri. 2000; 71(6): 1660s-4s.
7. Ouwehand AC, Salminen S, Isohawel E. Probiotics: an overview of beneficial effects. A. Van Leeuw. 2002; 82(1-4): 279-289.
8. Salminen S, Ouwehand A, Benno Y, Lee YK. Probiotics: How should they be defined? Trends Food Sci. Technol. 1999; 10(3): 107–110.
9. Mattila-Sandholm T, Myllarinen P, Crittenden R, Mogensen G, Fonden R, Saarela M. Technological challenges for future probiotic foods. Int. Dairy J. 2002; 12(2-3): 173-182.
10. Lucknow T, Delahunty C. Consumer acceptance of orange juice containing functional ingredients. Food Res. Int. 2004; 37(8): 805-14.
11. Panesar PS, Shinde C. Effect of storage on Synersis, pH, *Lactobacillus acidophilus* Count, *Bifidobacterium bifidum* Count of *Aloe vera* Fortified Probiotic Yoghurt. Current Research in Dairy Sciences. 2011; 4(1): 17-23.
12. Waller GR, Mangiafico S, Ritchey CR. A Chemical Investigation of *Aloe Barbadensis.* Miller, Proc. Okla. Acad. Sci. 1978; 58: 69-76.
13. Ranganna, S. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*, 1986; Tata Mc Graw Hill, India.
14. A.O.A.C. *Official Methods of Analysis of the AOAC*, 1995; 15th Ed. (Ed. S. Williams) Association of Official Analytical Chemists, Arlington,
15. Guzman-Gonzalez M, Moris F, Amigo L. Influence of skimmed milk concentrate replacement by dry dairy products in a low-fat set type yoghurt model system. Use of caseinates, co-precipitate and blended dairy powders. J. Sci. Food Agric. 79(8)1999; 80: **1117–1122**.
16. Nigam A, Ayyagari A. Calorimetric estimation of reducing sugars in the given sample by using dinitrosalicylic acid in *Lab Manual in Biochemistry, Immunology and Biotechnology*, Experiment-13. 2007; Tata McGraw-Hill Publishing Company Ltd., New Delhi 35-36.
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J. Biol. Chem. 1951; 193(1): 265-275.

18. Dororkar MP, Gupta AK, Khanuja SP, Rajaram S, Rita R. Molecular Assessment of Germplasm Diversity in *Aloe vera* Species Using RAPD and AFLP Analysis. Journal of Medicinal and Aromatic Plant Sciences. 2003; 25: 354-361.
19. Carpenter RH, McDaniel HR, McAnalley BH. Uses of Aloe Products in the Treatment of Chronic Respiratory Diseases, U.S. Patent, 1998; 5: 786 342.
20. Fox PF, Mc Sweeney P, Cogan TM, Guinee TP, *Fundamentals of cheese science*. 2000 1st Ed., Springer, New York, ISBN-10: 0834212609
21. Vahedi N, Tehrani MM, Shahidi F. Optimizing of Fruit Yoghurt Formulation and Evaluating Its Quality During Storage. Amer-Eurasian J. Agri. Envir. Sci. 2008:3 (6): 922-927.
22. Kailasapathy K, Rybka S. *Lactobacillus acidophilus* and *Bifidobacterium* Species: Their Therapeutic Potential and Survival in Yoghurt. Aust. J. Dairy Techol. 1997; 52: 28-35.
23. Christopher MD, Reddy VP, Venkateswarlu K. Viability during Storage of Two *Bifidobacterium bifidum* Strains in Set and Stirred Flavoured Yoghurts Containing Whey Protein Concentrates. Natural Product Radiance. 2009; 8(1): 25-31.
24. Abrahamsen RK, Rystad G. Fermentation of goat's milk with yoghurt starter bacteria "A review". J. Cult. Dairy Prod. 1991; 8: 20-26.
25. Salavador A, Fiszman SM. Textural and sensory characteristics of whole and skimmed flavoured set-type yoghurt during long storage. J. Soc. Dairy Technol. 87(12):4033-41.
26. Thomas TD, Mills OE. Proteolytic enzymes of dairy starter cultures. "A review". J. Food Microbiol. 1981; 46(3): 245-268.