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Effect of Storage on Syneresis, pH, *Lactobacillus acidophilus* Count, *Bifidobacterium bifidum* Count of *Aloe vera* Fortified Probiotic Yoghurt

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ABSTRACT

Probiotics are increasingly finding use as dietary supplements in processed foods. These are known for improving the host intestinal microbial balance and several potential health benefits. Interest in developing new probiotics formulations beckons the food and nutraceutical industry's interest to target general health and well-being of consumers. 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 and the effect of storage on syneresis, pH, *Lactobacillus acidophilus* count, *Bifidobacterium bifidum* count of *Aloe vera* fortified probiotic yoghurt was assessed for storage study. *Aloe vera* fortified probiotic yoghurt was produced using skim milk powder in *Aloe vera* juice and water blend, incubating at 37°C for 8 h. Bacterial counts were determined by pour plate technique whereas syneresis was determined according to standard procedures. Syneresis was increased from 4.7 to 8.3% (v/w), pH decreased from 4.03 to 3.91, *Lactobacillus acidophilus* count decreased from 39.7×10^9 cfu mL⁻¹ to 32.1×10^9 cfu mL⁻¹ and *Bifidobacterium bifidum* count decreased from 16.9×10^9 to 7.3×10^9 cfu mL⁻¹. Counts of both bacteria remain more than suggested value of more than 10^7 throughout the storage period. This study showed that the *Aloe vera* fortified probiotic yoghurt could be used as an adequate carrier of probiotic bacteria with bacterial counts more than suggested level.

Key words: Probiotic yoghurt, *Aloe vera*, *Lactobacillus acidophilus*, *Bifidobacterium bifidum*

INTRODUCTION

The role of fermented milk in human nutrition is well documented and the virtues of these products were known to man even during the ancient days of civilization. These products have long been an important component of nutritional diet. The medicinal and nutritional properties of various fermented foods have been experienced by several generations. An increasing number of dairy foods that carry a variety of health claims are appearing on the world food market. Milk, yoghurt, fermented milk, desserts, fruit juice and various kinds of cheese are among the major recent probiotic products (Saarela *et al.*, 2006; Aragon-Alegro *et al.*, 2007; Panesar *et al.*, 2009). Further improvement by addition of certain functional ingredients that can confer health effects on consumer is also desirable. Dairy foods seem to be fit naturally with probiotics because of the traditional association of beneficial fermentation bacteria and fermented dairy products (Sanders, 2000; El-Bakri and El-Zubeir, 2009; Panesar *et al.*, 2009).

Lactic acid bacteria including *lactobacilli* and *bifidobacteria* are the most common bacterial species considered as potential probiotics (Sanders, 1997). A number of health benefits have been claimed for *L. acidophilus* and *bifidobacteria* due to the ability of these organisms to establish them amongst the colonic microflora and they are increasingly being incorporated into dairy products. These bacteria have a beneficial influence on human health by improving the balance of intestinal microbiota and improving mucosal defenses against pathogens (Yesillik *et al.*, 2011). Additional health benefits include enhanced immune response, reduction of serum cholesterol, vitamin synthesis, anti-carcinogenic activity and anti-bacterial activity (Boylston *et al.*, 2004). To obtain the desired therapeutic effects, the yoghurt and probiotic bacteria must be available in sufficient numbers. It has been suggested that these organisms should be present in a food to a minimum level of 10^6 cfu g^{-1} (Robinson, 1987) of daily intake should be about 10^8 cfu g^{-1} (Gill and Rutherford, 2001; Silva *et al.*, 2004).

Recently there has been increasing trends to fortify the dairy product with herbal extract [tulsi leaf (*Ocimum sanctum*), pudina leaf (*Mentha arvensis*), coriander leaf (*Coriandrum sativum*), turmeric, *Mentha piperita*, *Ziziphora clinopodioides*, cinnamon and licorice] (Foda *et al.*, 2007; Chowdhury *et al.*, 2008; Al-Wabel *et al.*, 2008; Behrad *et al.*, 2009; Sarabi-Jamab and Niazmand, 2009) and other additives (Mumtaz *et al.*, 2008). If the probiotic products are fortified with the herbal additives then the formulated products can be unique and provide more health benefits. Moreover, it seems reasonable to assume that the beneficial effects of these probiotic bacteria can be expected only when viable cells are ingested (Nogueira *et al.*, 1998). Keeping in view, the present investigation was undertaken to study the effect of storage on syneresis, pH, *Lactobacillus acidophilus* count, *Bifidobacterium bifidum* count of Aloe vera fortified probiotic yoghurt.

MATERIALS AND METHODS

The study during the present was conducted during the period of May, 2010-Nov., 2010.

Procurement of cultures: The freeze-dried yoghurt cultures used for preparation of *Aloe vera* fortified yoghurt, viz., NDRI Yoghurt culture YH-3 containing *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and two probiotic cultures viz., *Lactobacillus acidophilus* and *Bifidobacterium bifidum* were obtained from National Dairy Research Institute, Karnal, India.

Media for differential enumeration: The MRS-salicin agar was used for the selective enumeration of *L. acidophilus* while, the viable numbers of *Bifidobacterium bifidum* were enumerated on MRS-NNLP agar (Laroia and Martin, 1991).

Preparation of low-fat set *Aloe vera* fortified probiotic yoghurt: The reconstituted skim milk for low-fat *Aloe vera* fortified set yoghurt was prepared by using 16.57 g skim milk powder in *Aloe vera* juice and water blend, 25 and 75 mL, respectively. The prepared reconstituted skim milk was heated or pasteurized properly at 82-85°C for 12-15 min. The reconstituted skim milk was cooled to 45°C. Inoculation was done using 3.46% (v/v) of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* cultures, then kept for incubation at 37°C for 8 h. After incubation the samples were kept under refrigerated condition at 4°C before performing physico-chemical, microbiological properties during entire storage period.

Determination of syneresis: Susceptibility of yoghurt to syneresis was determined by centrifuging 20 g of sample at 500 rpm for 5 min and weighing the supernatant (Guzman-Gonzalez *et al.*, 2000). Then measuring the amount of supernatant recovered (% v/w). Percent syneresis calculated as:

$$\% \text{Syneresis} = \frac{\text{volume of supernatant}}{\text{weight of sample}} \times 100$$

Microbiological analyses: Microbial count was carried after subsequent serial decimal dilutions using agar plates of *L. acidophilus* were incubated aerobically at 37°C for 72 h (Dave and Shah, 1996) while the plates of *B. bifidum* were incubated at 37°C for 72 h under anaerobic conditions (Laroia and Martin, 1991) using pour plate technique. After incubation, typical colonies of bacteria were counted with the help of Darkfield Quebec Colony Counter by AO Scientific Instruments Model no. 3327 [Quebec colony counter]. Plates with 30-300 colonies were used in counting and the counts were averaged for three replicates and expressed as cfu mL⁻¹.

All the experimentations were performed in triplicate and the mean values are reported.

RESULT AND DISCUSSION

The effect of storage on physico-chemical and microbiological characteristics of the *Aloe vera* fortified probiotic yoghurt are discussed below:

Effect of storage on syneresis: The effect of storage on syneresis revealed that the value of syneresis increased with the storage time (Fig. 1). The initial value of syneresis for fresh yoghurt was found to be 4.7% (v/w) which after storage of 28 days increased to 8.3% (v/w). There was slow increase in the syneresis up to initial period (14th day) of storage, however, thereafter a rapid increase in this value was observed. An increase in syneresis value from 5.7% (v/w) to 6.8% (v/w) was observed up to 21st day. The results are in conformation with the research of previous workers (Fox *et al.*, 2000), wherein they stated that rate of syneresis is directly related to the acidity and therefore is inversely related to pH.

Effect of storage on pH: During storage, the pH in fresh sample decreased from 4.03 to 3.91 (Fig. 2). Thus there was decrease in pH constantly throughout the storage period. This decrease might be attributed to the utilization of residual carbohydrates by viable microorganisms and

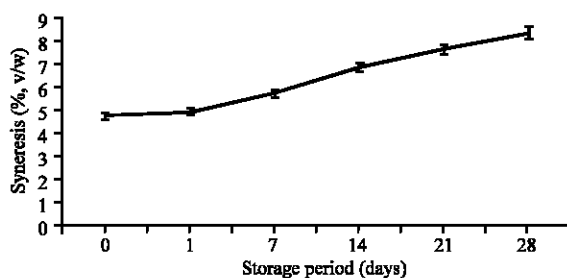


Fig. 1: Effect of storage on syneresis of *Aloe vera* fortified yoghurt. Bars indicate the standard deviation from triplicate determinations

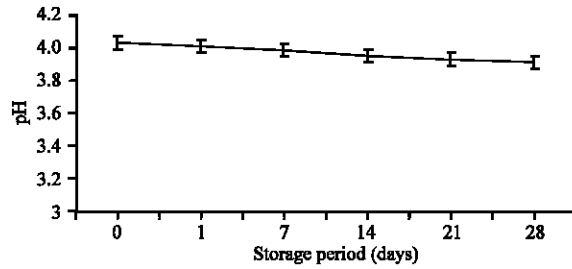


Fig. 2: Effect of storage on pH of *Aloe vera* fortified yoghurt. Bars indicate the standard deviation from triplicate determinations

production of lactic acid, small amounts of CO₂ and formic acid from lactose. Vahedi *et al.* (2008) reported that the decrease in pH is due to the microorganism's activity, whereas Kailasapathy (2006) stated that post-acidification, during storage, is due to β-galactosidase which is still active at 0-5°C. In this case, pH may decrease to less than 4.2. Some researchers suggested that the drop in pH during storage period is due to residual enzymes produced by starters during fermentation (Christopher *et al.*, 2009).

Effect of storage on *Lactobacillus acidophilus* count: The changes in the viable counts of probiotic bacteria from manufacturing to storage during four weeks of the *Aloe vera* fortified probiotic yoghurt were monitored during manufacture and storage of yoghurt for 28 days at 4°C (Fig. 3). It was observed that the strains of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* shown good viability in combination with the basic yoghurt cultures. However, the counts after 28 days remained more than suggested level (Medina and Jordan, 1994). During storage, the *Lactobacillus acidophilus* count in fresh sample decreased from 39.7×10⁹ to 32.1×10⁹ cfu mL⁻¹. *Lactobacillus acidophilus* had shown sharp decrease after 21st day and shown good viability for 21 days. The results are in close conformation with Martin (1994) who found that probiotic were acid tolerant and can survive in sufficiently higher numbers to remain viable in cultured dairy products even during storage. This point could be beneficial to manufacturer in using these strains on industrial scale, to produce functional products with better viability of beneficial microorganisms.

Effect of storage on *Bifidobacterium bifidum* count: The effect of storage on *Bifidobacterium bifidum* count has been given in Fig. 4. During storage, the *Bifidobacterium bifidum* count in fresh sample decreased from 16.9×10⁹ to 7.3×10⁹ cfu mL⁻¹. It can be depicted from the results that *Bifidobacterium bifidum* count shown sharp decrease throughout the storage period but its count is more than suggested value of 10⁷ cfu gm⁻¹. This dramatic loss termed may be attributed to hydrogen peroxide produced by the starter *lactobacilli*.

The decrease in *L. acidophilus* and *B. bifidum* may also be due to antagonist relationship between yoghurt bacteria and probiotic strains and dissolved oxygen content may directly affected survival of *L. acidophilus* during storage (Bari *et al.*, 2009). According to Champagne *et al.* (2005), oxygen affects the probiotic cultures in two ways. The first is a direct toxicity to cells. Certain probiotic cultures are very sensitive to oxygen and die in its presence, presumably due to the intracellular production of hydrogen peroxide. The second way the oxygen effects the probiotic cultures is indirect, when oxygen is in the medium certain cultures, particularly *L. delbrueckii*,

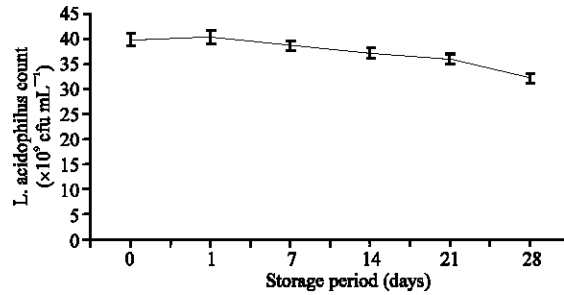


Fig. 3: Effect of storage on *Lactobacillus acidophilus* count of *Aloe vera* fortified yoghurt. Bars indicate the standard deviation from triplicate determinations

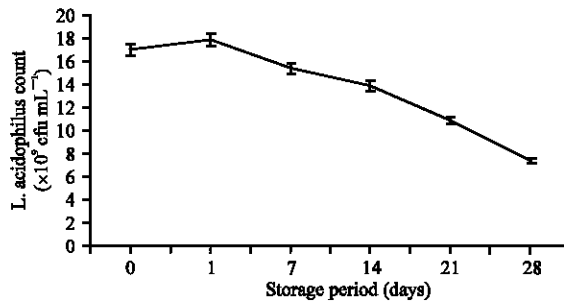


Fig. 4: Effect of storage on *Bifidobacterium bifidum* count of *Aloe vera* fortified yoghurt. Bars indicate the standard deviation from triplicate determinations

excrete peroxide in the medium and a synergistic inhibition of *bifidobacteria* by acid/peroxide has been demonstrated. The antagonism among the bacteria used in starter cultures caused by antimicrobial substances such as bacteriocins may also decrease the numbers of any sensitive organisms present in a product and starter culture (Shah, 2000).

CONCLUSION

Aloe vera fortified probiotic yoghurt has shown good viability of probiotic cultures i.e., *Lactobacillus acidophilus* and *Bifidobacterium bifidum* during storage. Optimized sample of *Aloe vera* fortified probiotic yoghurt remained with populations of both probiotic bacteria above 10^9 cfu mL $^{-1}$ during the whole storage period. Therefore, the *Aloe vera* fortified yoghurt has shown good potential for commercial exploitation.

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