A PROMISING FUNCTIONAL DAIRY FOOD USING PROBIOTICS WITH KALMEGH

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ABSTRACT

Kalmegh extract has well known nutritional and medicinal values. A probiotic yoghurt fortified with Kalmegh extract (KE) was manufactured using Lactobacillus acidophilus together with Bifidobacterium bifidum as a probiotic bacterial strains. Kalmegh extract was prepared and added at a concentration of 6% to Buffalo's skim milk. The effect on syneresis, pH, TTA and sensory properties of the final product, as well as, the viability of the LAB were evaluated during 28 days of storage at refrigeration temperature. Lactobacillus acidophilus counts decreased from 36x10^8 cfu/ml to 14x10^8 cfu/ml and Bifidobacterium bifidum counts decreased from 100x10^9 cfu/ml to 5x10^9 cfu/ml, which is more than the recommended value of more than 10^7 throughout the storage period. Thus kalmegh fortified yoghurt could be used as a functional dairy food.

KEYWORDS: Kalmegh, probiotics, Lactobacillus acidophilus, Bifidobacterium bifidum, fortified yoghurt, functional food.

INTRODUCTION

Probiotic bacteria are defined as "living micro-organisms", which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition” (Guarner and Schaafsma, 1998). Two such species include Lactobacillus acidophilus and Bifidobacterium bifidum. They are pH and bile resistant, survive passage through and colonize in the gastrointestinal tract. They exhibit antagonistic effects towards enteropathogenic bacteria and are able to relieve from diarrhoea without causing adverse systemic immune or inflammatory outcomes.
These probiotics are known to enhance immune function, reduces cholesterol levels, reduces the risk of diarrhoea, reduces the risk of eczema, relieves lactose intolerance symptoms and exhibits antitumorigenic activity (Marteau et al., 2001, Calder & Kew, 2002, Wright et al., 2002, El-Shenawy et al., 2012, Mishra et al., 2008). Because of these health benefits, efforts have been devoted to incorporate bifidobacteria and Lactobacillus into dairy products and yoghurt containing probiotic cultures can prove a successful vehicle. Now a days, claims about the ability of foods, including herbs and spices, to lower disease risk or to enhance the quality of life continue to held interest on our lives (Kaefer C.M. and Milner J.A., Gupta P, 2015). However, properties of yoghurt such as pH and presence of oxygen and antimicrobial activity of herbal extracts can create a suboptimal environment for some probiotic species, reducing viability to below $10^6$-$10^8$ colony forming units (CFU)/mL, which is the minimum accepted amount required to produce health benefits on the host (Reid et al., 2001, Hekmat S et al., 2009, Hemsworth J et al., 2011, Behestipour H et al., 2012 and Cimo A et al., 2013). Therefore, it is important for the probiotic product fortified with herbal extract to serve the effective dose of probiotics required for health benefits, and for viable counts to be maintained throughout a standard product shelf-life of 28 days.

In the present study Andrographis paniculata nees (Acanthaceae) is used for fortification of milk which is a medicinal plant traditionally used for the treatment of anti-inflammatory, antibacterial, antioxidant, antiparasitic, antispasmodic, antidiabatic, anti carcinogenic, antipyretic, antidiarrhoeal, hepatoprotective, nematocidal, anti- HIV and several infectious diseases ranging from malaria to dysentery. The plant is widely used in ayurvedic and homeopathic systems of medicines. The medicinal value of this plant is due to the presence of active ingredients viz., andrographolide and neoandrographolide which are derivatives of diterpenoids. It prevents oxidative damage and inhibits binding to toxic metabolites to DNA. The present study investigated the effects of the herb on yoghurt fermentation and viability of probiotic bacteria during refrigerated storage.

**MATERIALS AND METHODS**

Procurement of cultures: the freeze dried culture of *Bifidobacterium bifidum* was obtained from NCBI, India and the locally isolated culture of *Lactobacillus acidophilus* from UGC project.
Preparation of extracts
Powder of dry leaves of kalmegh was obtained from local resources. The powdered herbs (10g) were soaked in 100ml of distilled water and left overnight at 70°C. The suspension was then centrifuged (2000 rpm; 15 min), and the supernatant was filtered.

Herbal yoghurt preparation
Homogenized and pasteurized buffalo milk was purchased from the local supermarket. Herb extract (6%v/v) was added in 100ml of milk. Inoculation was done using *Bifidobacterium bifidum* and *Lactobacillus acidophilus* culture (3% v/v). Incubation was carried out at room temperature for 18 hours then the samples were stored in refrigerator at 4°C.

Determination of ph and tta
The pH and TTA of yoghurts were determined during storage at 4°C. Yoghurt sample (1g) was mixed with distilled water (1:1), and the pH was measured using a pH meter. TTA was determined by titrating yoghurt sample and distilled water (1:9) mixture with 0.1N NaOH using a 0.1% Phenolphthalein as colour indicator. The amount of acid produced during fermentation was calculated as follows:

\[
\text{TTA} = \text{Dilution factor (10) x V NaoH x 0.1N x 0.009 x 100%}
\]

where V is volume of NaoH required to neutralize the acid. (Behrad *et al.*, 2009).

Enumeration of probiotic bacteria
 Enumeration of *Lactobacillus* spp and *Bifidobacterium* spp. was carried out by aseptically mixing yoghurt sample (1ml) with 9ml of distilled water and subsequently serial dilution technique was followed. Microbial count was carried out in agar plates of *Lactobacillus* spp. and *Bifidobacterium* spp. incubated anaerobically (CO₂ incubator) at 37°C for 24-48 hours. Lactobacillus MRS agar (HiMedia) plates were used. For enumeration of *Lactobacillus* spp where as for enumeration of *Bifidobacterium* spp, BSC Propionate Agar (HiMedia) was used. Viable microbial count was calculated as follows:

\[
\text{cfu/ml} = \text{cfu/plate x dilution factor}
\]

(cfu : colony forming units)

Sensory properties evaluation
Yoghurt samples were evaluated for their sensory properties (appearance, aroma, consistency and flavour) on a 7-point hedonic scale (7-excellent, 6-liked a lot, 5-liked, 4-liked and did not liked, 3-disliked, 2-much disliked and 1-unacceptable) performed by expert judges selected
and local resources. Yoghurt samples were presented in two-digit coded white plastic containers and tasted 20 minutes after leaving refrigerator.

**Determination of syneresis**
An amount of 10 g of the yoghurt sample was placed into centrifuge tube and centrifuged at 500rpm for 5 min and weighing the supernatant (guzman-gonzalez et al., 2000). The weight fraction of the supernatant liquid was used as index of whey syneresis. Then measuring the amount of supernatant recovered (% v/w)

\[
\% \text{ syneresis} = \frac{\text{volume of supernatant}}{\text{weight of sample}} \times 100
\]

**RESULTS AND DISCUSSION**
**EFFECT OF STORAGE ON PH**
During the storage the decrease in pH from 5.8 to 5.2 was observed constantly throughout the storage period (fig.1). This decrease might be attributed to the utilization of residual carbohydrates and production of lactic acid, small amounts of CO₂ and formic acid from lactose by viable microorganisms. (Panesar and Shinde, 2011).

![Change in pH during storage](image)

**Figure 1.** Effect of storage on pH

**EFFECT OF STORAGE ON SYNERESIS**
Syneresis was found to increase from 38.5 to 74.0% during 28 days of storage as shown in fig.2. The increase in syneresis may be due to increase in acidity i.e. decrease in pH throughout the storage period. (Fox et al., 2000)
Effect in Total Titrable Acid (TTA) of yoghurts during storage
The TTA of all yoghurts increased from the initial values of 0.45% to 0.81% by day 28 of storage (fig.3). The increase in acids can be attributed to continued production of organic acids by LAB during refrigerated storage.

SENSORY PROPERTIES
The score of sensory properties viz. appearance, aroma, consistency and flavour on 7point hedonic scale are depicted in fig.4. Mean value of sensory properties scores are within the range of acceptance which depict it to be consumable.
Effect of storage on *Lactobacillus acidophilus* count

The changes in viable count of probiotics i.e., *Lactobacillus acidophilus* and *Bifidobacterium bifidum* was monitored during manufacture and storage of Kalmegh fortified probiotic yoghurt for 28 days in refrigerator at 4°C. During first week of storage an increase in count was observed from $36 \times 10^8 \text{cfu/ml}$ to $55 \times 10^8 \text{cfu/ml}$ and then it decreases from $55 \times 10^8 \text{cfu/ml}$ to $14 \times 10^8 \text{cfu/ml}$. It showed good viability till 21 days of storage and then a sharp decrease in viable count was noticed (fig.5). This result are stastically at par with the findings of aloevera yogurt (Panesar and Shinde, 2011).

Effect of storage on *Bifidobacterium bifidum* count

The changes in viable count of *Bifidobacterium bifidum* during manufacture and storage of Kalmegh fortified probiotic yoghurt for 28 days in refrigerator at 4°C are shown in fig.5.
During the storage period the bacterial count decreases from 100x10^9 cfu/ml to 5x10^9 cfu/ml. It showed good viability till 14 days of storage afterward a major decrease was noticed.

The viable count of *Bifidobacterium bifidum* was found to be much more than *Lactobacillus acidophilus* throughout the storage period. The results indicate that kalmegh extract may support the *Bifidobacterium* spp. more than *Lactobacillus* spp. The decrease in *Lactobacillus acidophilus* count may be due to antagonistic relationship between the probiotic strains and low dissolved oxygen content (Bari *et al.*, 2009). However the counts of both the probiotics are more than the suggested value of 10^7 cfu/ml throughout the storage period. The results are in confirmation with Martin (1994) who found that probiotic can survive in sufficiently higher numbers to remain viable in cultured dairy products even during storage.

**CONCLUSION**

Kalmegh fortified probiotic yoghurt has shown good viability of probiotic cultures i.e., *Bifidobacterium bifidum* and *Lactobacillus acidophilus* during storage period of four weeks. Viable counts of both the probiotics are more than the suggested value of 10^7 cfu/ml throughout the storage period. The results suggest the potential of kalmegh fortified yoghurt for its commercialization as a functional food providing prevention from various infections and diseases.

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REFERENCES


