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RESEARCH ARTICLE

Survivability of probiotics co-encapsulated with prebiotics in pasteurised flavoured milk during storage

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Abstract : The pasteurised flavoured milk supplemented with encapsulated probiotics with prebiotic gave lower colony counts than non encapsulated probiotics during refrigerated storage. There was no spoilage observed in both flavoured milks. This suggests that encapsulated probiotics are not released in flavoured milk and upon ingestion all are expected to be released in the intestine together with prebiotics.

Key words : Microbial analysis in flavoured milk, Survivability of flavoured milk.

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INTRODUCTION

In India, milk is widely consumed for its excellent nutritive value. Flavoured milk is one of the milk product which has a good consumer acceptance as a refreshing and nourishing milk beverage. Probiotics "For Life" are living, health-promoting microbial food ingredients that have a beneficial effect on humans (Chuayana *et al*., 2003). Prebiotics are classified as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improve host health" (Gibson and Roberfroid, 1995). Several factors have been reported to affect the viability of probiotics in dairy products, being important to deliver health beneficial effects. It has confirmed that probiotic strains exhibit poor survivability in traditional fermented dairy products (Lourens-Hattingh and Viljoen, 2001). Different approaches have been attempted to increase the resistance of the probiotic bacteria against adverse conditions it may encounter in GIT and product. Microencapsulation technique is currently receiving considerable attention to enhance the survivability Mortazavian *et al.* (2007). The co-encapsulation of probiotic with prebiotic is reported to improve the survival rate of probiotics (Chen *et al*., 2005). An *in vitro* study has been conducted to study the survivability of co-encapsulated probiotics (*L. paraplantarum* 321 and *B. bifidum* 235) with prebiotics (FOS) in pasteurised flavoured milk during eight days of refrigerated storage.

RESEARCH METHODOLOGY

The *L. paraplantarum* 321 and *B. bifidum* 235 strains were obtained from NDRI, Karnal and cultured in MRS broth to produce freeze dried powder. Alginate beads were produced by incorporating 1×10^7 cfu/g of probiotic bacteria with 3% of commercial prebiotic FOS and (2%) sodium alginate utilising a modified extrusion technique originally reported by Chen *et al*. (2005) using a micro-encapsulator. Probiotic flavoured milk was prepared according to the method of Sadaghdar *et al*. (2012) with some modifications using encapsulated probiotics with prebiotics. The method to determine the viable

counts of the encapsulated probiotic bacteria added in flavoured milk was evaluated according to the method followed by Chen *et al*. (2005). The viable count of non encapsulated probiotic bacteria in flavoured milk samples were evaluated using MRS agar medium by serial dilution method, incubated at 37^0C for 48 h. Enumeration was carried out for eight days at refrigerated storage (4^0C) at 4 day intervals. Flavoured milk samples (10 ml each) were centrifuged at 2000 rpm for 5 minutes and the leftover sediment was measured in millimetres to find sedimentation in flavoured milk samples. The data were subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) 15th version and the treatment means were compared with Duncan multiple range test.

RESULTS AND DISCUSSION

The experimental findings obtained from the present study have been discussed in following heads:

Microbial analysis of flavoured milk :

Mean probiotic counts (log_{10} cfu/g) on 4th and 8th day of T_3 and T_5 flavoured milk groups, respectively were significantly ($p<0.05$) higher than T_2 and T_4 groups, respectively. It may be due to addition of prebiotics (FOS) which may have acted as a substrate for non encapsulated probiotics to grow. Earlier Shin *et al.* (2000) concluded that *Bifidobacterium* Bf-1 and Bf-6 showed greater retention when grown in the presence of FOS in skim milk. Gibson and Roberfroid (1995) also stated that prebiotics selectively stimulate probiotic strains. The viable bacterial counts in both encapsulated groups were lesser than their non encapsulated

Fig. 2 : Viability counts of non encapsulated *B. bifidum* 235 in flavoured milk

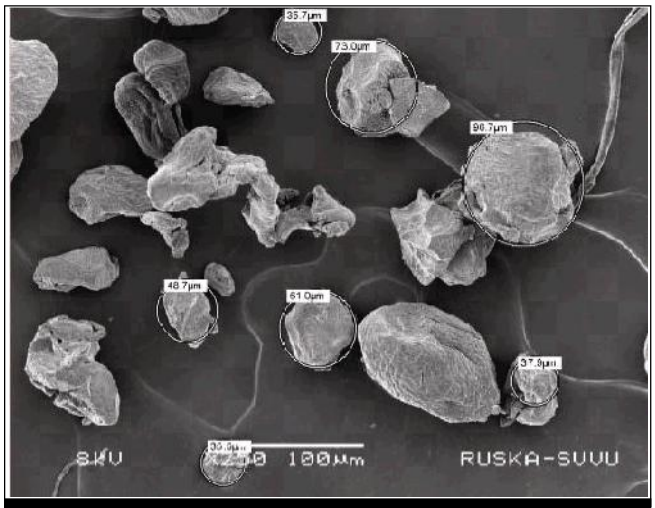

Fig.1 : Scanning electron microscopy showing varying sizes of alginate microcapsule

Fig. 3 : Viability counts of co-encapsulated *B. bifidum* 235 in flavoured milk

Table 1 : Effect of different treatments and storage periods on mean probiotic bacteria counts (log 10 cfu/g) of flavoured milk

Treatment	Initial day	Fourth day	Eighth day
T_2 (Encapsulated *L. paraplantarum* 321 with FOS)	7.56±0.04	7.85a±0.05	7.19a±0.06
T_3 (Non encapsulated *L. paraplantarum* 321 with FOS)	7.60±0.08	8.55b±0.05	7.80b±0.05
T_4 (Encapsulated *B. bifidum* 235 with FOS)	7.47±0.04	7.80a±0.08	7.28a±0.09
T_5 (Non encapsulated *B. bifidum* 235 with FOS)	7.52±0.04	8.68b±0.04	7.85b±0.08

Each mean value is obtained from three replications, ab values indicates of significance of values at P=0.05, respectively

Fig. 4 : Viability counts of non encapsulated *L. paraplantarum* 321 in flavoured milk

Fig. 5 : Viability counts of co-encapsulated *L. paraplantarum* 321 in flavoured milk

groups, respectively. It may be due to reduced availability of prebiotics and other nutrients to probiotic bacteria in the microcapsules. But, probiotic bacteria survived well and counts were in accordance to the levels recommended by FAO/WHO (10^6-10^7cfu/g). The result shows that encapsulation improved the viability of probiotic bacteria. Previous reports by Hansen *et al.* (2002) suggests that survivability of micro-encapsulated *Bifidobacterium longum* Bb-46 using alginate as a coating material was better than free cells during refrigerated storage in milk with 2% fat. The result shows that co-encapsulation of probiotic bacteria with alginate and prebiotic FOS protected probiotic bacteria in flavoured milk (Rokka and Rantamaki, 2010).

Sedimentation test :

There was no sedimentation of microcapsules in flavoured milk supplemented with micro-encapsulated probiotics during storage. It may be due to small size of alginate beads which were in the range of 35.7-96.7 μm (Fig. 1).

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