



## Probiotic Viability of Freeze Dried Synbiotic Microcapsules in Skim Milk Powder at Ambient Storage Condition

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### Abstract

This study investigated the probiotic viability of synbiotic skim milk powder under ambient storage condition and its effect on the quality characteristics of skim milk powder for 60 days with 6% of moisture content and 37% of relative humidity. Oil emulsion technique was used for the microencapsulation of *Lactobacillus casei* MTCC 1423 with high resistance maize starch and inulin in various percentage (w/w) combinations. The slurry of synbiotic microcapsules by microencapsulation was subjected to freeze drying that resulted as dried powder of synbiotic microcapsules. Synbiotic microcapsules H0I3 showed 99% probiotic survivability in skim milk powder during 60 days of ambient storage condition and rate of mortality was 0.000277833/day. Maximum probiotic viability in H0I3 synbiotic skim milk powder affects the biochemical characteristics. ANOVA showed significant effect of probiotic viability on biochemical characteristics of synbiotic skim milk powder i.e. lactic acid, protein content, and fat content at  $p < 0.05$  level. Physical characteristics i.e. moisture content, bulk density and flowability remained constant.

**Keywords:** prebiotic, probiotic, synbiotic, *Lactobacillus casei*, microcapsules

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### Introduction

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host<sup>1</sup>. This definition has the following characteristics. A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota (FAO/WHO, 2002). Prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota (FAO/WHO, 2007).

Mixture of pro- and prebiotics is synbiotic which beneficially affects the host by improving the survival and implementation of live microbial dietary supplements in the gastrointestinal tract by selectively stimulating the growth and/or by activating the metabolism of health promoting bacteria and thus improving host welfare.

According to this approach, a food or food supplement will include both the live cells of the beneficial bacteria and a selective substrate; the idea being that the beneficial bacterial cells that survive their transit through the stomach can grow quickly and competitively because of the presence of the selective substrate and establish their predominance (Roberfroid et al., 2000).

A growing interest has arisen in the inclusion in dried foods of viable probiotics of long-term shelf life at ambient temperatures and of survival in sufficient numbers to provide a health benefit to consumers. To that end, drying techniques to obtain dehydrated probiotic organisms in a viable state have proven useful; and although freeze drying (Lyophilization) has been the most widely used for long time preservation of probiotic (Ananta et al., 2005).

Development of technique for packaging of probiotic cells by prebiotic material gives higher probiotic viability is called microencapsulation. Microencapsulation has been investigated for improving the viability of microorganisms in both dairy products and the GI tract (Krasaekoopt et al.,

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2003). It has been defined as the incorporation of food ingredients, enzymes, oils, bacterial cells or other nutraceuticals into small capsules that can release their contents at controlled rates under specific conditions and that protect their contents from degradation by the detrimental factors in their environment (Desai et al., 2005). The purpose of microencapsulation of probiotics is to stabilize and maintain viability during storage (O'Riordan et al., 2001), to protect against harsh gastro-intestinal environment (Muthukumarasamy et al., 2006) and controlled release in the colon (Reid et al., 2005). High resistant maize starch is the well known prebiotic material which is not digested by pancreatic enzymes (amylases) in the small intestine. It reaches the colon where it is fermented by probiotics (Sajilata et al., 2006; Anal and Singh, 2007). This specificity provides a good enteric delivery characteristic to efficiently release the probiotics into the large intestine. Moreover, by its prebiotic functionality, high resistant maize starch can be used by probiotic bacteria in the large intestine (Mortazavian et al., 2008). Finally, resistant starch provides an ideal surface for the adherence of the probiotic cells to the starch granules (Anal and Singh, 2007) and this enhances probiotic delivery in a viable and metabolically active state to the intestine (Crittenden et al., 2001).

## Material and Methods

### Materials

Freeze dried culture of *Lactobacillus casei* MTCC 1423 used as probiotic was provided by Microbial Type Culture Collection and Gene Bank, Chandigarh, India. Spray dried skim milk powder was purchased from Krishna Dairy Foods, India. Microbiological media were collected from Himedia, India and chemicals were collected from SRL chem., and Fischer scientific, India. Sun flower oil was collected from local retailer in Chennai. Inulin and High maize resistance starch were obtained from Quality Food Ingredients, Chennai.

### Methods

#### Cultivation of probiotic biomass

*Lactobacillus casei* 1423 was grown in MRS medium (Zhao et al. 2002). A pure lyophilized 5 mg culture of *Lactobacillus casei* was suspended with 2 ml sterile peptone water 0.1% (w/v) and incubated at 37°C for 24 h. When the liquid became turbid, 98 ml MRS broth medium was added, and the culture was incubated at 37°C for 24 h to make the mother solution. The mother solution was centrifuged (Remi RC-8 India) at 2000 rpm for 5 minutes. The supernatant was collected and centrifuged at 4500 rpm for 30 min followed by the collection of bacterial pellet. The pellet was washed with 10 ml sterile physiological saline, and centrifuged again at 4500 rpm for 30 min. The

process of centrifugation and re-suspension in saline was repeated for three times and pellet obtained after the third centrifugation was suspended in 10 ml sterile physiological saline and used as the core material for microencapsulation.

### Microencapsulation

Numerous techniques for microencapsulation are available. oil emulsion technique was used in this work for microencapsulation of *Lactobacillus casei*. It is a suitable method for microencapsulation of probiotic bacteria with microcapsules less than 100 µm diameter. It was found to have no detrimental effects on the mouth feel of food products (Sultana K., 2000; Sheu et al., 1993; Aziz Homayouni et al., 2007; Nasrin Moayednia et al., 2009). Eight types of synbiotic microcapsules were developed on the basis of difference in prebiotic composition according to table 1. Complete process of microencapsulation describe in flow diagram of microencapsulation.

### Freeze drying

The freeze-drying process was performed (Higl et al. 2007). Aliquots of 50 g aqueous microcapsule-slurry were transferred into glass vials with an inner diameter of 25 mm and dried using a bench-scale freeze dryer (Lyodel freeze dryer, LYO1550, India). The samples were initially pre freeze at -40°C in the pre freezer bath at atmospheric pressure. After 1 h the sample allow for drying process was initiated by reducing the vial pressure to 0.370 mbar and raising the shelf temperature to +10°C. The following freeze-drying process was carried out under these conditions for 4 h.

### Enumeration of probiotic population in Synbiotic microcapsules as CFUs/g

Freeze drying of microencapsulated slurry gave a powder of synbiotic microcapsules followed by enumeration of probiotic count (CFU/g) by plate count on MRS (De Man, Rogosa and Sharpe medium). 0.1 g of synbiotic microcapsules were suspended in the 9.9 ml sterile 0.02M citric acid solution in tubes at room temperature. The dissolved samples were vortexed at high speed to break up particles and several dilutions of sample were prepared. 0.1 ml of the samples was plated in duplicate on MRS agar. Colony forming units (CFU) were determined after 48 h of incubation at 37°C by cubic colony counter (Harrigan et al., 1976).

### Incorporation of synbiotic microcapsules in skim milk powder

Skim milk powder was allowed to autoclave in a hermetic glass bottle at 121°C for 15 minutes for killing all the microbial cells present in it. Synbiotic skim milk powder was developed by addition of 1 g synbiotic microcapsules into autoclaved skim milk powder and allowed to aseptically blending by a laboratory scale blender at 1000 rpm for 15min and followed by packaging of sample in

silver pouch with  $30 \pm 2$  micron thickness and 145 sq.cm in area. 100 g of sample was stored in each pouch. It was stacked in a plastic transparent box at  $25 \pm 5^\circ\text{C}$ .

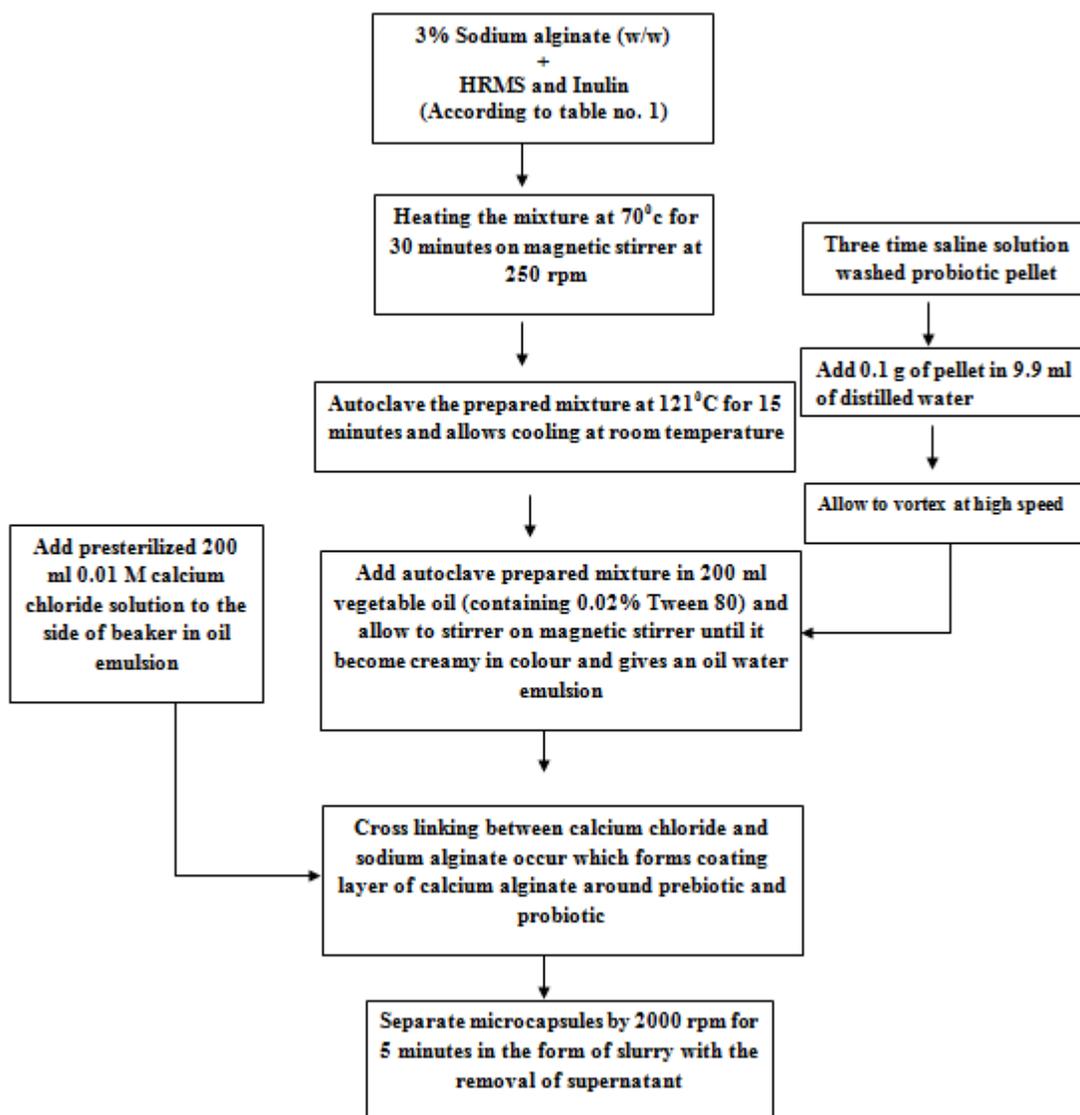
### Physico-chemical analysis

Storage study of synbiotic skim milk powder was done with biochemical analysis for protein, acidity, fat and physical analysis for moisture, bulk density, flowability. Protein content was determined as described by Pyne (1932), lactic acid content was analyzed as Titratable acidity (Morris B

Jacobs, 1999), fat, bulk density, flowability, moisture content were analyzed as described by Kim *et al.*, (2009).

### Statistical analysis

All the tests were performed in triplicate. ANOVA was used to study significant of probiotic viability on the chemical, physical characteristic of skim milk powder during ambient storage of skim milk powder at  $p < 0.05$  level. Microsoft excel 2007 statistical tool was used to perform statistical analysis in this study.



**Figure 1.** Flow chart showing steps of oil emulsion microencapsulation for *L. casei*.

**Table 1.** Composition of prebiotic in synbiotic microcapsules for microencapsulation and notation.

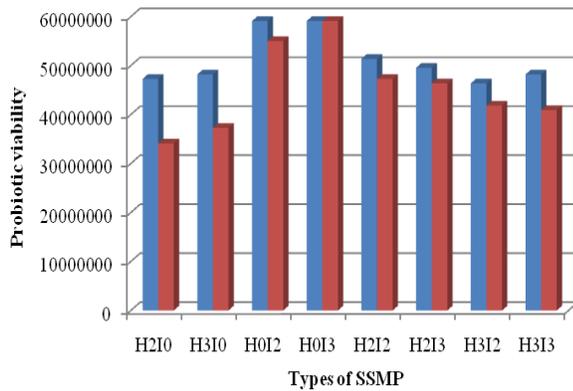
Composition of synbiotic microcapsules	H2I0	H3I0	H0I2	H0I3	H2I2	H2I3	H3I2	H3I3
Inulin %	0	0	2	3	2	3	3	2
High resistance maize starch %	2	3	0	0	2	2	2	3
Sodium alginate %	3	3	3	3	2	3	3	3
Probiotic cells pellet %	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Calcium chloride 0.1M (ml)	200	200	200	200	200	200	200	200
Vegetable oil (ml)	200	200	200	200	200	200	200	200

Notation: “H” High resistance maize starch and “I” Inulin

## Results

### Probiotic viability

Initial probiotic viability of eight different types of freeze dried synbiotic microcapsules was varying. They were  $4727292 \times 10^3$ ,  $4818194 \times 10^3$ ,  $5909105 \times 10^3$ ,  $5909106 \times 10^3$ ,  $513637 \times 10^4$ ,  $4954554 \times 10^3$ ,  $4636371 \times 10^3$ , and  $4818192 \times 10^3$  in H2I0, H3I0, H0I2, H0I3, H2I2, H2I3, H3I2, and H3I3 respectively. Probiotic viable count in synbiotic skim milk powder was changed after 60 days of ambient storage condition.



**Figure 2.** Probiotic viability in synbiotic skim milk powder on 1<sup>st</sup> day and after 60<sup>th</sup> days of ambient storage condition.

### Probiotic survivability

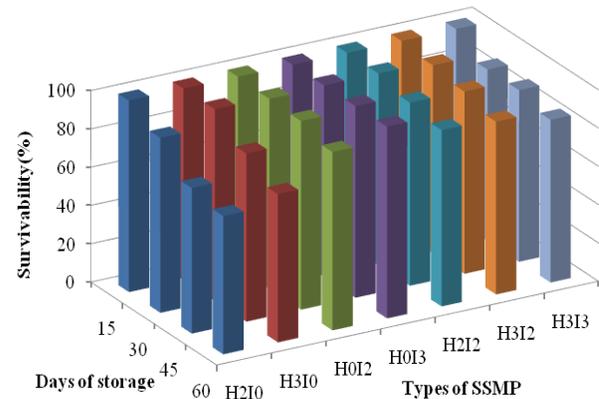
Probiotic survivability of synbiotic skim milk powder was affected during the ambient storage condition. It is defined as the ratio of initial population of probiotic in synbiotic skim milk powder and population at a time of analysis in terms of percentage.

$$\text{Survivability (\%)} = (N/N_0) \times 100$$

where, N is probiotic population at a time of analysis after storage and  $N_0$  is initial population of synbiotic skim milk powder.

Synbiotic skim milk powder which had H2I3 type of synbiotic microcapsules shown maximum probiotic

survivability after 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup> and 60<sup>th</sup> days of storage period were



**Figure 3.** Survivability of probiotic (%) in eight different types of synbiotic skim milk powder during ambient storage condition.

### Relationship between storage time and viable count

During storage colony count decrease as a function of time and fit reasonably well to an exponential equation  $N_{(t)} = N_1 \cdot e^{-R_m \cdot t}$

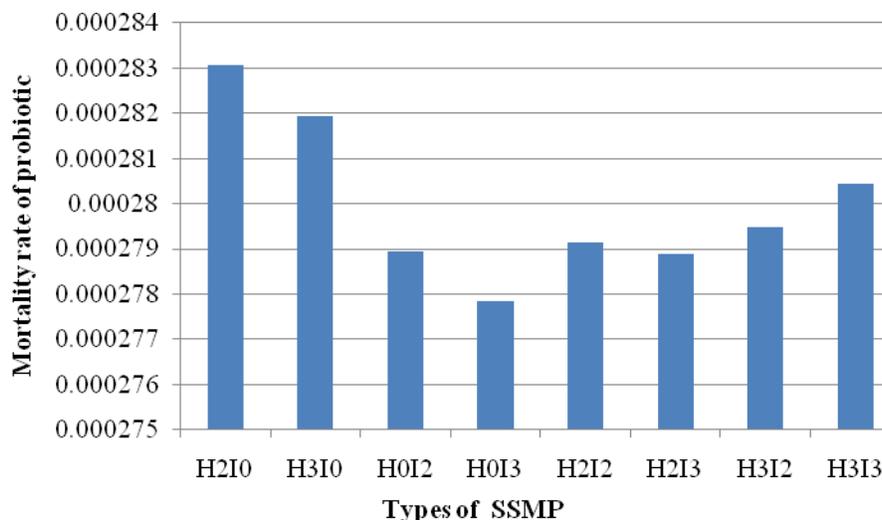
where, N is the viable count of bacteria (CFU/g of SSMP),  $N_1$  is the bacterial at the beginning of storage (after incorporation of synbiotic microcapsules in skim milk powder) and t is the storage time in the days.  $R_m$ /day is the rate of mortality that expresses the rate at which viability of bacteria decays during storage (King *et al.*, 1974; Mitic *et al.*, 1998). Mortality rate of probiotic was least in H0I3 type of synbiotic skim milk powder. It was 0.000277833/day.

### Physicochemical changes in synbiotic skim milk powder (SSMP)

During ambient storage condition of synbiotic skim milk powder the change in probiotic viability and changes in biochemical characteristic respectively affect the level of quality as a view of self life. There were no changes in physical characteristic of skim milk powder during 60 days

of ambient storage condition. Multiple linear regression was established between the independent probiotic viability and dependent variable protein content, acidity, and fat content of skim milk powder. Synbiotic skim milk powder H0I3

type gave 100% probiotic viability during 60 days of storage. Changes in physicochemical property of synbiotic skim milk powder significant at  $P < 0.05$  level but changes in physical property were not significant.



**Table 2.** Changes in physicochemical property of H0I3 synbiotic skim milk powder after 15<sup>th</sup>, 30<sup>th</sup>, 45<sup>th</sup>, and 60<sup>th</sup> days of storage.

Flowability (Angle Of Repose)	Bulk Density	Moisture Content	Titration Acidity	Protein	Fat
35.00 ± 0.72	0.54 ± 0.01	6.09 ± 0.00	1.09 ± 0.06	25.93 ± 0.06	0.70 ± 0.01
35.00 ± 0.72	0.54 ± 0.01	6.09 ± 0.05	1.11 ± 0.01	25.90 ± 0.10	0.70 ± 0.01
35.00 ± 0.72	0.54 ± 0.01	6.09 ± 0.05	1.09 ± 0.05	25.89 ± 0.01	0.69 ± 0.01
35.00 ± 0.72	0.54 ± 0.01	6.09 ± 0.05	1.24 ± 0.07	25.70 ± 0.44	0.69 ± 0.01

### Discussion

Viability of probiotic in food and food products is a challenge for the food processing industry. To maintain the viability of probiotic at refrigeration condition can be cost effect as economic view of consumer and producers. Invention of microencapsulation technique is very effective to maintain the viability of probiotic in food carrier. The use of microencapsulation technique to enhance the survival rates of probiotic microorganisms in connection with an application in food can be considered to be promising. (Champagne *et al.*, 1994; Park and Chang, 2000). Viability of encapsulated probiotic cells depend on the physicochemical properties of the capsules. In fact, the type and the concentration of the coating material and initial cell numbers and bacterial strains are some basic parameters which are important to for maintaining the probiotic viability (Chen *et al.*, 2007). Inulin composition of synbiotic microencapsules affect the viability of probiotic in skim milk powder with the increasing of inulin composition

in synbiotic development increased the probiotic viability in this work 3% inulin in synbiotic microcapsules showing 99% of the probiotic viability at 60 days of ambient storage condition. Alginate hydro gels are extensively used in cell encapsulation (Rowley *et al.*, 1999) and calcium alginate is preferred for encapsulating probiotics because of its simplicity, non-toxicity, biocompatibility and low cost (Krasaekoopt *et al.*, 2003). 3% sodium alginate was used for the coating of probiotic with prebiotic. resistant starch is an ideal surface for the adherence of the probiotic cells to the starch granules (Anal and Singh, 2007) and this can enhance probiotic delivery in a viable and a metabolically active state to the intestine (Crittenden *et al.*, 2001). Various composition of high resistance maize starch was used for the encapsulation of probiotic which had least property to maintain probiotic viability in compare to inulin during ambient storage within the synbiotic skim milk powder.

## Conclusion

Survivability (%) and mortality rate of probiotic in symbiotic skim milk powder depend upon the prebiotic composition (HRMS and inulin) containing 3% inulin content in symbiotic microcapsule showed 99.9% probiotic viability. It was proved to be more efficient when compared with alone HRMS content and other combinations of HRMS with inulin. Hence higher the probiotic viability in symbiotic skim milk powder was directly affected the protein, fat, lactic acid content at 60 days of ambient storage condition.

## Acknowledgements

We express our gratitude to Dr. P. Gurumoorthi, Department of Food Process Engineering, SRM University, Kattankulathur, Chennai for the immense support of this study.

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