Influence of Prebiotic Composition on Probiotic Survivability in Calcium Alginate Coated Synbiotic Microcapsules at Thermal Incubation

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Abstract: The aim of this study is to improve the thermal resistivity of prebiotic on probiotics, using various combinations of high resistance maize starch and inulin in the form of synbiotic microcapsules. Lactobacillus casei MTCC 1423 was used as probiotic strain. Oli emulsion technique was used for the microencapsulation of Lactobacillus casei with prebiotic (high resistance maize starch and inulin) as thermal protective agent and calcium alginate as coating material. The freeze drying process produced agglomerated powder of synbiotic microcapsules. Eight types of synbiotic microcapsules were exposed to 45, 50, 55, 60, 65 and 70°C for 15, 30, 45 and 60 min which revealed that the effect of prebiotic composition on the thermal resistance property of probiotics on probiotics was significant (p<0.05) as analyzed using ANOVA. Multiple linear regression proved that inulin provides higher heat resistance to probiotics than that of high resistance maize starch when used in combination. Synbiotic microcapsule type H2I3 was found with highest probiotic survivability 40.36% during thermal incubation. The rate of probiotic death (k) and heat resistance property (D) were found to be 0.056 day⁻¹ and 17.67±839 min, respectively at 70°C when exposed for 60 min.

Key words: Microencapsulation, probiotic, prebiotic, synbiotic, Lactobacillus casei, India

INTRODUCTION

Probiotics are the live microbial feed supplements that beneficially affect the host health by improving its intestinal microbial balance. The whole concept of probiotics is not new and in fact, they have been consumed by human beings in the form of fermented foods for thousands of years (Kopp-Hoolihan, 2001; Ranadheera et al., 2010). Probiotic bacteria cannot thrive well in digestive tract without of its prebiotic (Cruz et al., 2010). The combination of probiotic with its prebiotic gives synergistic effect on host health (Morelli et al., 2003). It has been suggested that adding non-digestible food ingredients known as prebiotics to certain foods may increase the viability of bacteria passing through the gastrointestinal tract and thus exert a beneficial effect on human health (Chow, 2002; Foeks et al., 1999; Iyer and Kailasapathy, 2005; Khalf et al., 2010) combination of both prebiotic and probiotic termed as synbiotic.

A number of intrinsic and extrinsic factors influence the survival of probiotics in foods during processing and storage. It is important to consider these factors at all stages from additions of the probiotic in the food to delivery of the probiotic to the gut of the consumer. These include manufacturing processes, food formulations and matrices, packaging materials and environmental conditions in the supply chain and storage.

Higher levels of viable probiotic micro-organisms 10⁸-10⁹ CFU g⁻¹ of the product are recommended in probiotic foods for better efficacy in regulating beneficial effects. It is a great challenge for the food processors to maintain probiotic viability during processing as well as storage. During processing of food temperatures >45°C are detrimental for survival of free probiotic cells. The higher temperature and shorter time period ranging from minutes to hours at 45-55°C leads to reduction of the probiotic viability (Doleyres and Lacroix, 2005).

Microencapsulation is a newest emerging technology and most efficient method of chemical and mechanical process for packaging of prebiotic and probiotic as synbiotic microcapsules which isolates the probiotic cells from the adverse environmental conditions (Lee et al., 2004; Shah and Ravula, 2000; Gardiner, 2000; Heidebach et al., 2010) and reduces the cell losses. Microencapsulation provides the physical protection to probiotic bacteria by prebiotic against detrimental conditions during processing of food (Anal and Singh, 2007). Physical protection of probiotic bacteria in the form of microcapsules is a new concept that does not give any detrimental effects on organoleptic property of food (Homayouni et al., 2007; O’Regan and Mulvihill, 2007).
Many techniques of microencapsulation are available but in this study, oil emulsion technique was used. It is a suitable method for encapsulation of probiotic bacteria with smaller in size <100 μm (Critenden et al., 2006; Vidyalakshmi et al., 2009). Inulin and high resistance maize starch are legally classified as prebiotic food or food ingredients in all countries (Kolida et al., 2002; Coussenement, 1999).

Aim of this study was to investigate the effect of prebiotic contents on probiotic viability in symbiotic microcapsules at various time and temperature combination. It is expected to be useful for process designing in the development of thermally processed probiotic products.

**MATERIALS AND METHODS**

In this study, *Lactobacillus casei* MTCC 1423 was used as probiotic strain supplied by Microbial Type Culture Collection and Gene Bank Chandigarh, India in freeze dried form. All microbiological media were collected from Himedia and Sodium alginate collected from SRL, India. The sun flower oil was procured from the local market. Inulin and high resistance maize starch used as prebiotic were obtained from SD fine Chem. Ltd., India.

**Bacterial growth and harvesting:** Freeze dried pure culture of *Lactobacillus casei* was activated by inoculating a loop of culture in the MRS-broth at pH 6.8 and incubated at 37°C for 72 h. The probiotic cells in late-log phase were harvested by centrifugation at 4500 rpm for 10 min and the supernatant was discarded. Collected pellet was washed three times in sterile, 0.9% saline solution and again centrifuged. Finally, pellet was collected and used for microencapsulation process (Chen et al., 2006).

**Microencapsulation:** Oil emulsion technique was used for the production of symbiotic microcapsules (Sheu and Marshall, 1993; Homayouni et al., 2007; Moayednia et al., 2009). All the solutions and glassware required for microencapsulation process were sterilized at 121°C for 15 min. A mixture of 3% (w/w) sodium alginate and prebiotics, i.e., high resistance maize starch and inulin (Table 1) was prepared in double distilled water followed by heating of the mixture at 70°C for 20 min and allowed to cool at room temperature (Olivas et al., 2007). On the other hand, 0.1% suspension of washed pellet of *Lactobacillus casei* was prepared in double distilled water. About 10 mL suspension of *L. casei* was added in already prepared 100 g mixture of sodium alginate and prebiotic at room temperature. It was mixed using magnetic stirrer for 10 min for equal distribution of probiotic cells into the mixture. It was lead to formation of symbiotic mixture.

It was added to presterilized 200 mL of sun flower oil containing 0.02% tween 80 and stirred at 400 rpm using magnetic stirrer for 20 min till it was emulsified and appeared creamy. It was followed by addition of 200 mL of 0.1 M calcium chloride solution quickly along the side of the beaker then cross linkage between sodium alginate and calcium chloride occur which formed calcium alginate coated symbiotic microcapsules. Calcium alginate coated symbiotic microcapsules soon settled down to the bottom of the beaker in chloride solution. Complete process of cross linking took 45-50 min.

The oil phase was removed from the top with aspirator and microcapsules were harvested from chloride solution by gentle centrifugation at 200 rpm for 5 min with the removal of supernatant. The microcapsules were washed with 5% of saline solution containing 5% glycerol and again centrifuged at same condition. Thus, slurry of calcium alginate coated symbiotic microcapsules was obtained. This was stored at 4°C for 24 h.

**Freeze drying of symbiotic microcapsules slurry:** The freeze drying process was performed by manifold freeze dryer (Lyodel, LY01550, India) (Higl et al., 2008). Aqueous slurry of calcium alginate coated symbiotic microcapsules were transferred to 100 mL round bottom flask and pre-freezed to -40°C in the pre freezer at atmospheric pressure for 2 h.

It was allowed to freeze drying for 6 h with reduction of pressure on the round bottom flask to 10⁻² m bar and raising the shelf temperature of sample +10°C. After completion of freeze drying, dry agglomerates of symbiotic microcapsules were obtained.

**Table 1: Composition of prebiotic in symbiotic microcapsules for microencapsulation and notation**

<table>
<thead>
<tr>
<th>Composition of symbiotic microcapsules</th>
<th>H2O</th>
<th>H3O</th>
<th>H2O2</th>
<th>H3O2</th>
<th>H2O3</th>
<th>H3O3</th>
<th>H2O4</th>
<th>H3O4</th>
</tr>
</thead>
<tbody>
<tr>
<td>High resistance maize starch (%)</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Inulin (%)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Sodium alginate (%)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Probiotic cells pellet (%)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Calcium chloride 0.1 M (mL)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Vegetable oil (mL)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
</tbody>
</table>

H: High resistance maize starch; I: Inulin
Thermal incubation of symbiotic microcapsules: Thermal incubation of symbiotic microcapsules was done in 15 mL test tube. A suspension of peptone water and microcapsules was prepared. Total 1 g of freeze dried symbiotic microcapsules was added to each test tubes containing, 9 mL of 0.1% (w/v) sterilized peptone water and suspension was agitated for 15 sec using high speed cyclomixer. It was incubated in water bath at 45, 50, 55, 60, 65 and 70°C temperature for 15, 30, 45 and 60 min.

Enumeration of thermal incubated probiotic bacteria: After cooling of thermal incubated suspension containing tubes, 1 mL suspension was removed after 15 sec of cyclomixing and added to sterilized 9 mL of 0.02 M citric acid solution. It was allowed to cyclomixing at high speed to break up coated layers of microcapsules. Several dilutions were prepared by using 0.1% peptone water 0.02 M citric acid solution used as solvent of calcium alginate which releases probiotic cells from calcium alginate coated symbiotic microcapsules without affecting their viability (Denish et al., 1995). About 1 mL of the samples from each dilution tube was inoculated in sterilized Petriplates poured with 12 mL of MRS agar medium at 45°C. Selected plates had 25-250 colonies as described by Houghby et al. (1992) after 48 h of incubation at 37°C. The colonies were enumerated using cubic colony counter and calculated as colony forming units (cfu) per gram of symbiotic microcapsules.

Survivability of probiotic bacteria: Survivability of Lactobacillus casei expressed in percentage was determined as initial probiotic count (cfu g⁻¹) of symbiotic microcapsules before thermal incubation and probiotic count (cfu g⁻¹) after thermal incubation:

\[ \text{Survivability (\%)} = \left( \frac{N_i}{N_f} \right) \times 100 \]

Where:
- \( N_i \) = Probiotic viability (cfu g⁻¹) of symbiotic microcapsules before thermal incubation
- \( N_f \) = Probiotic (cfu g⁻¹) of symbiotic microcapsules after thermal incubation

Statistical analysis: All the experiments were performed in duplicates. The multiple linear regression was used to estimate average relationship between time and temperature to predict viability of probiotic in calcium alginate coated symbiotic microcapsules. Two way ANOVA tests were performed to signify the effect of the time, temperature and prebiotic composition factor on the viability of probiotic after heat treatment of symbiotic microcapsules. MINITAB software (Version 15) was used for statistical analysis.

RESULTS AND DISCUSSION

The different combinations of prebiotic yielded various amounts of symbiotic microcapsules after freeze drying of the slurry. Higher composition of prebiotic incorporation during microencapsulation gave higher yield of symbiotic microcapsules. Initial probiotic count (cfu g⁻¹) of freeze dried symbiotic microcapsule was varying (Table 2).

Microencapsulation of Lactobacillus paracasei and Bifidobacterium lactis improves the survivability after incubation at low pH (Heidebach et al., 2010). 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 maintain the probiotic viability (Chen and Chen, 2007).

Viability of probiotic decreased with increase in temperature and/or time of the thermal incubation. Therefore, the mortality rate was different in particular types of microcapsules. Difference in probiotic viability after thermal incubation was different in specific types of symbiotic microcapsules. In this study, high resistance maize starch 2% and inulin 3% proved to be a good combination of prebiotics to provide thermal resistance to probiotic.

Two factor ANOVA showing significant effect of time and temperature on the probiotic survivability (%) in eight different types of symbiotic microcapsules at p<0.05. Multiple linear regression revealed the effect of time and temperature on the probiotic survivability as follows:

\[ Y_1 = 215 - 2.04 X_1 - 0.775 X_2 \]

where, \( Y_1 \) is probiotic survivability (%) at \( X_1 \) time and \( X_2 \) temperature combination of thermal incubation of eight types of symbiotic microcapsules.

Regression analysis of probiotic survivability versus high resistance maize starch and inulin had a significant effect for survivability of probiotic during thermal incubation as follows:

<table>
<thead>
<tr>
<th>Microcapsule Type</th>
<th>Yield after freeze drying (g)</th>
<th>Initial cfu g⁻¹ of SM</th>
<th>Log cfu g⁻¹ of SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>H20</td>
<td>6.03</td>
<td>4.81 x 10⁶</td>
<td>9.67 x 10⁶</td>
</tr>
<tr>
<td>H30</td>
<td>6.97</td>
<td>4.81 x 10⁶</td>
<td>9.68 x 10⁶</td>
</tr>
<tr>
<td>H21</td>
<td>5.23</td>
<td>5.9 x 10⁶</td>
<td>9.77 x 10⁶</td>
</tr>
<tr>
<td>H22</td>
<td>6.72</td>
<td>5.9 x 10⁶</td>
<td>9.77 x 10⁶</td>
</tr>
<tr>
<td>H23</td>
<td>7.02</td>
<td>5.1 x 10⁶</td>
<td>9.71 x 10⁶</td>
</tr>
<tr>
<td>H31</td>
<td>9.41</td>
<td>4.9 x 10⁶</td>
<td>9.69 x 10⁶</td>
</tr>
<tr>
<td>H32</td>
<td>8.23</td>
<td>4.6 x 10⁶</td>
<td>9.66 x 10⁶</td>
</tr>
<tr>
<td>H33</td>
<td>8.50</td>
<td>4.81 x 10⁶</td>
<td>9.68 x 10⁶</td>
</tr>
</tbody>
</table>

233
Table 3: Rate of probiotic death (K) min⁻¹ of H213 probiotic microcapsules under thermal incubation with given time and temperature combination

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time of thermal incubation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>45</td>
<td>0.200000053</td>
</tr>
<tr>
<td>50</td>
<td>0.2080808091</td>
</tr>
<tr>
<td>55</td>
<td>0.201659336</td>
</tr>
<tr>
<td>60</td>
<td>0.20276137</td>
</tr>
<tr>
<td>65</td>
<td>0.205225721</td>
</tr>
<tr>
<td>70</td>
<td>0.207543196</td>
</tr>
</tbody>
</table>

Table 4: Heat resistance property of probiotic H213 probiotic microcapsules in terms of decimal reduction time (D) min at given time and temperature of combination

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time of thermal incubation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td>45</td>
<td>4.999999885</td>
</tr>
<tr>
<td>60</td>
<td>4.931303568</td>
</tr>
<tr>
<td>70</td>
<td>4.818274071</td>
</tr>
</tbody>
</table>

Stumbo et al., 1975; Pflug, 1987; Casolaro, 1988). According to equation of exponential decay:

\[ N_t = N_0 e^{-kt} \]

Where:
- \( N_t \) = The cfu g⁻¹ of probiotic microcapsules after thermal incubation at specific temperature for time (t)
- \( N_0 \) = The initial cfu g⁻¹ of probiotic
- \( k \) = The death rate (min⁻¹)

The Decimal reduction time (D) is widely used to heat resistance property of microorganisms exposed to higher temperature. It is expressed in min:

\[ D_t = \frac{1}{K} \log \left( \frac{N_0}{N_t} \right) \]

Synbiotic microcapsules type H213 provided highest the probiotic survivability after the thermal incubation of 70°C and 60 min time temperature combination. Rate of probiotic inactivation (k) was found least and heat resistance property was found highest on the basis of D value at various time temperature combination of thermal incubation (Table 3 and 4).

CONCLUSION

Microencapsulation process provides a way to develop thermal resistance property in probiotics by prebiotics. These types of prebiotics helped to develop
thermal resistant probiotic microcapsules. H213 was the highest thermal resistant probiotic microcapsules. Highest probiotic survivability was maintained at 70°C for 60 min of thermal incubation and proved 2% high resistance maize starch and 3% inulin is the best combination of prebiotics to maintain probiotic survivability during thermal processing. Therefore, inulin alone is not effective as thermal protective agent for probiotic.

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REFERENCES


