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Characteristics of synbiotic spray dried powder of litchi juice with *Lactobacillus plantarum* and different carrier materials

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ABSTRACT

Spray drying of probiotic *Lactobacillus plantarum* in litchi juice with varied proportion of maltodextrin, fructooligosaccharide (FOS) and pectin was performed to determine the effect on cell viability. The spray dried powders were investigated for physical, physicochemical and toxicological properties and survivability of cells in simulated gastric and intestinal digestion. The results showed that coating material comprising of maltodextrin 10% (w/v) and 5% (w/v) FOS gave highest yield and survivability of the encapsulated bacteria. This composition of the coating material also showed good solubility, low water activity, satisfactory colour, uniform particle size with smooth surface. Powder coated with 10% (w/v) maltodextrin and 5% (w/v) pectin showed reduced powder properties and cell viability even though had the highest glass transition temperature. Maltodextrin at 10% (w/v) and FOS at 5% (w/v) levels in the coating material can be considered as the optimal carrier material composition for protection of the bacterial cells from damage by adverse environmental conditions of spray drying and for possessing desirable powder characteristics. This combination also provided protection to bacterial cells in gastric environment and also enhanced their growth in simulated digestive system.

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1. Introduction

Probiotics are live microorganisms having health benefiting properties on the host when administered in adequate amounts (FAO/WHO, 2002). Most of the probiotic products are dairy based. Non-dairy products like probiotic fruit juice or dehydrated fruit juice could be alternative probiotic foods for the consumers who are either intolerant to dairy based products or are strictly vegetarian (Vasudha & Mishra, 2013). Probiotication of fruit juices has gained the interest of researchers as fruit juices are considered healthy and consumption is almost regular. However, direct addition of probiotic culture to fruit juice results in loss of viability because of the acidic environment, high dissolved oxygen concentration and insufficient amounts of free amino acids and peptides. Such juices also develop altered flavour profile that is not desirable (Granato, Branco, Nazzaro, Cruz, & Faria, 2010). The probiotic microorganisms present in food should survive in significant numbers of at least 10^6–10^8 CFU/g (Chávez and Ledeboer, 2007). However, processing conditions like temperature, pH, pressure, acidity, gastric acid, and bile salts decrease their viability. In order that probiotics are released in the gastro-intestinal tract in sufficient numbers, they are microencapsulated (Ivanovska et al., 2014). Encapsulation of probiotics ensures active maintenance even under high water activity during storage (Weinbreck, Bodnar, & Marco, 2010). Spray drying method of microencapsulation yields stable microparticles of homogenous size distribution and viability of probiotics is affected by the inlet and outlet temperatures used in spray drying (Kingwatee et al., 2015). Wall materials like maltodextrin, oligosaccharide, starch and proteins are used for microencapsulation (Ezhilarasi, Indrani, Jena, & Anandharamakrishnan, 2013). Of late, addition of prebiotics along with probiotic foods has gained the interest of researchers for their bifidogenic effect (Roberfroid, 2007) and thermoprotection (Krasaekoopt & Watcharapoka, 2014) and in the subsequent storage period. The ability of prebiotics to decrease the moisture content and water activity in the microencapsulates is desirable for powder stability (Anandharamakrishnan & Padma, 2015). Fructooligosaccharide (FOS), inulin, gum arabic and pectin are prebiotics of interest in

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foods (Ezhilarasi et al., 2013). FOS is a low molecular weight short chain carbohydrate, which though studied extensively in food applications (Saad, Delattre, Udaci, & Schmitter, 2013), has been found to create problems in dried products because of stickiness. Stickiness due to FOS is attributed to its low glass transition temperature (Rajam, Bharath Kumar, Prabhasankar, & Anandharamakrishnan, 2015). On the other hand, maltodexrin has high glass transition temperature (Desai & Park, 2005; Ersus & Yurdagel, 2007) and has a protective effect on encapsulated material. By binding a low glass temperature material into a high glass transition material, the overall mixture ends up with a weight fraction-based glass transition temperature. In other words, higher concentration of maltodextrin in the coating material will yield a non rubbery glassy amorphous state. Pectin also has a high glass transition temperature (Lai, Sung, & Chien, 2000). Litchi (*Litchi chinensis* Sonn.) is an important tropical fruit and excellent source of vitamin C (40–90 mg/100 g) (Menzel, Xuming, & Chengming, 2005). Litchi also contains 21.6 g/100 g of total sugar (Haq & Rab, 2012), 0.37 g/100 g titratable acidity (as citric acid) (Sun, Liang, Xie, Lei, & Mo, 2010), 0.8 mg/g total phenolic compounds (Dajanta, Apichartsrangkoon, & Somsang, 2012) and more than 40 aroma volatile compounds (Wu, Pan, Qu, & Duan, 2009). Fortification of litchi juice with probiotics in powdered form could produce an important product for the different food industries. Kingwatee et al. (2015) reported the spray drying of litchi juice with *Lactobacillus casei* using maltodextrin and inulin as coating materials. Synbiotics, i.e., combination of probiotic and prebiotic agents improves the survival of bacteria in the upper gastrointestinal tract and enhance their effect in the large bowel (Chen, Chen, Liu, Lin, & Chiu, 2005). The objective of the present study was to evaluate the effect of spray drying of prebiotics differing in glass transition temperature along with maltodextrin and *Lactobacillus plantarum* in litchi juice on the properties of spray dried powder and probiotic viability. Whether microencapsulation of *L. plantarum* MTCC2621 and litchi juice within symbiotic coating materials affects the viability of probiotics when exposed to conditions simulating the passage through the gastrointestinal tract was also studied.

2. Materials and methods

2.1. Materials

Ripe sweet variety of litchi (*Litchi chinensis Sonn.*) fruit was purchased from the local fruit market at Tezpur, Assam during the season. Fructooligosaccharides (FOS) from chicory (Sigma) was used. Pectin having 7% methoxyl content and 80% galacturonic acid and maltodextrin having 20DE and all other chemicals and media were purchased from Himedia Laboratories, India. Human embryonic kidney cell line (HEK 293) was procured from National Centre for Cell Science (NCCS), NCCS Complex, University of Pune Campus.

2.2. Lactobacillus strain and growth condition

Lactobacillus isolate, *Lactobacillus plantarum* MTCC2621 was obtained from Microbial Type Culture Collection and Gene (MTCC) (Chandigarh, India). From this culture, stock solution was prepared by adding sterile glycerol (50% v/v) to the activated culture. The glycerol stock culture was stored at −20 °C in sterile screw cap tubes. The identity of all the probiotic bacteria was confirmed using biochemical methods described by Shah and Lankaputhra (1997). The probiotic organism was grown individually and inoculated into 10 mL sterile de Man Rogosa and Sharp (MRS) broth and incubated at 37 °C for 2 days under aerobic condition. The cells were harvested by refrigerated centrifuging (Sigma, Germany) at 1500×*g* for 15 min at 4 °C. Before inoculation into fruit juices, the harvested cells were washed twice with sterile saline water (0.85% w/v sodium chloride) to remove any residual MRS. The cell pellets were diluted with 0.85% sterile saline water to get a bacterial concentration of 10^{11} CFU/mL.

2.3. Litchi juice preparation

The fruits were peeled and pitted. The juice after extraction in a household juicer (Philips) was strained through a muslin cloth and pasteurized at 90 °C for 1min with constant stirring. Subsequently, the juice was cooled down to 25 °C. The pasteurized juice had pH 3.65 ± 0.26 and TSS of 14.6 ± 0.3 °Brix. The juice was inoculated with the probiotic bacteria to get population of 10^{11} CFU/mL.

2.4. Spray drying conditions

Spray drying was performed using a spray drier (Lab Plant, UK) having nozzle size of 0.1 mm. The drying conditions of the experiment were: 27 °C feed temperature, 40–60 mL/min feed rate, 14 psi atomizing pressure and 100–115 °C inlet temperature. Pasteurized litchi juice was mixed with 15% (w/v) coating material and divided into four lots. The first lot had only 15% (w/v) maltodextrin (≤20 DE); the second lot contained 10% (w/v) maltodextrin and 5% (w/v) pectin; third lot contained 10% (w/v) maltodextrin and 5% (w/v) fructooligosaccharide (FOS); fourth lot had 5% (w/v) maltodextrin, 5% (w/v) pectin and 5% (w/v) FOS and the lots were designated as M, MP, MF and MPF, respectively. The total soluble sugar (TSS) of the litchi juice was adjusted to 11 °Brix (total solid concentration 0.1 g/L) by appropriate dilution with sterile distilled water as the pure extract was too viscous to be spray dried. Homogenization of the probiotic juice and coating material was done using a magnetic stirrer (LaboTech) just before spray drying. The obtained powders were kept in airtight containers and stored at refrigeration condition (4 ± 2 °C).

2.5. Quantification of the viable encapsulated cells

The protocol of Ananta, Volkert, and Knorr (2005) was followed for enumeration of probiotics to determine cell viability in spray dried powder during refrigerated condition The L. *plantarum* microcapsule powders as well as the drying media aliquots were suspended and diluted in phosphate buffer saline under constant shaking for 10 min at room temperature to ensure complete dissolution of the powders. Serial dilutions in PBS were carried out and subsequently pour plated on molten MRS agar and the plates were incubated at 37 °C for 72 h under aerobic conditions. Enumeration of the bacteria was performed in triplicate following the standard plating methodology and the total counts of the viable bacteria were expressed as log colony forming units per gram (log CFU/g). The viability of the bacteria after the spray-drying process was calculated according to the formula below.

$$\%\ \text{viability} = \frac{N}{No} \times 100$$

2.6. Yield

The yield of the spray drying process was calculated by taking into consideration the total solid content of the juice and the carrier material and weight of the final dry powder.

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