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Lactobacillus rhamnosus GG encapsulation by spray-drying: Milk proteins clotting control to produce innovative matrices

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ABSTRACT

A well-known probiotic strain, *L. rhamnosus* GG, was encapsulated by spray-drying in milk water-insoluble matrices upon reconstitution in hot water by exploiting and controlling the clotting reaction of milk proteins during the process. The feed solution, composed of probiotic bacteria and milk proteins, was or not subjected to the action of chymosin, a proteolytic enzyme. To optimize microencapsulation efficiency, different outlet air temperatures were tested (55, 70 and 85 °C). After spray-drying, small microparticles were recovered for further characterization. All drying conditions led to excellent bacterial survival rates (<0.5 log reduction) whereas only the highest outlet air temperature allowed the production of microparticles with acceptable moisture contents (<7%) to ensure storage stability. Finally, enzymatic cleavage of milk proteins by chymosin before atomization led to matrices presenting innovative functionalities when microparticles are reconstituted with water: rehydration or dispersion in cold (8 °C) or warm (40 °C) water, respectively.

1. Introduction

Consumer requests for healthy food products that prevent illnesses strongly increased at the beginning of the 21st century. The interest for functional food, among which probiotic food, has rapidly grown these last years (Abd El-Salam and El-Shibiny, 2015). Probiotic bacteria are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). Probiotics have been incorporated in many food products, such as dairy products that usually constitute suitable probiotic carriers (Burgain et al., 2011; Granato et al., 2010). Nevertheless, after introduction in the product, a loss of viable cells during preservation has often been observed (Blanchette et al., 1996; Ding and Shah, 2008; Shah et al., 1995; Tripathi and Giri, 2014). For example, during refrigerated storage, five commercial yogurts containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* presented a constant decrease in the viable counts of these two strains (Shah et al., 1995). In dairy products, pH decrease and accumulation of inhibitory substances, like lactic acid produced during fermentation, were the main factors identified for the loss of probiotics viability in yogurt (Shah, 2000). This viability decrease was also observed in other dairy product such as cheeses (Amine et al., 2014; Blanchette et al., 1996; Gobbetti et al., 1998) and in a huge number of other food products, such as dark chocolate (Laličić-Petronijević et al., 2015) and fruit juices (Ding and Shah, 2008; Saarela et al., 2006). During digestion, a decrease in viable probiotics in the gastrointestinal transit is well documented (Burgain et al., 2013; Jantzen et al., 2013; Pinto et al., 2015) meaning that few or no microorganisms would be able to reach the intestine to exert their activity, presumably because of the low gastric pH and the presence of bile salts (Charteris et al., 1998; Cook et al., 2012). For example, a high loss of viable cells is observed for some strains (*L. rhamnosus* GG, *L. reuteri* and *Bifidobacterium* BB-12) in simulated gastric digestion, but these bacteria remain stable under the physicochemical conditions of the small intestine (Burgain et al., 2013; Charteris et al., 1998; Jantzen et al., 2013; Pinto et al., 2015).

To exert their probiotic activity, bacteria need to be viable when reaching the intestine. Microencapsulation is undeniably one solution. This technology is used to maintain probiotic viability from

Abbreviations: CFU, Colony Forming Units; CMP, Caseino-macro-peptide; IMCU, International Milk Clotting Units; SEM, Scanning Electron Microscopy; MRS, Man, Rogosa, Sharpe broth culture.

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their processing up to their consumption and their passage through the gastrointestinal tract by entrapping and protecting sensitive living cells (De Prisco and Mauriello, 2016). Many microencapsulation technologies are successful in encapsulating probiotic bacteria, e.g., spray-drying, emulsion, coacervation, extrusion, fluid bed or gel-particle technologies (Burgain et al., 2011; Krasaekoopt et al., 2003; Martín et al., 2015).

Among these encapsulation methods, gelation properties of milk proteins are sometimes exploited (Burgain et al., 2013; Heidebach et al., 2009). To this end, milk-clotting enzymes, as rennet or transglutaminase, are required to form a resistant matrix. Rennet is a proteolytic enzyme that is capable of hydrolyzing the κ-casein from the casein micelle surface and releases the caseinomacropeptide. By releasing these hydrophilic fragments, repulsive forces between caseins disappear allowing thereby the close approach of the micellar caseins via calcium bond formation. Upon heat treatment, micelle aggregation occurs until forming a gel (Dalgleish and Corredig, 2012). Different authors encapsulated probiotic cells (*L. rhamnosus* GG, *B. lactis* Bb12, *L. paracasei* F19) in milk water-insoluble microparticles by exploiting these enzymatically-induced gelation properties (Burgain et al., 2013; Heidebach et al., 2009). The main drawback of these processes is the use of an emulsification method that is no straight forward for the food industry.

The most common method used to encapsulate probiotic bacteria in food industry is spray-drying. This technique presents advantages of low cost, reproducibility and rapidity and is a useful way to incorporate probiotics into dairy products (Gardiner et al., 2002). Numerous studies already reported the use of spray-drying to encapsulate probiotic cells, but the major disadvantage of this technology is the use of high air temperatures causing a decrease in bacterial survival. The ability to survive to process conditions also depends on the probiotic strain. For example, spray-dried *Bifidobacterium breve* and *Lactobacillus acidophilus* presented a survival rate of only 26% and 76%, respectively (Maciel et al., 2014; Picot and Lacroix, 2004) and for *Lactobacillus reuteri*, a decrease of two log of the bacterial population was reported after spray-drying (Ananta et al., 2005). demonstrated that the reduced viability of *L. rhamnosus* GG is linked to cellular membrane damage and the percentage of membrane damage increased with the outlet air temperature, showing that the outlet air temperature should be carefully selected for improving the encapsulation of living probiotic cells by spray-drying.

In the present work, enzymatically-induced gelation properties of milk proteins were for the first time exploited to encapsulate *L. rhamnosus* GG by spray drying instead of an emulsification technique. The clotting reaction control was able to create matrices presenting new functionalities: water-insoluble upon reconstitution in hot water or water-soluble upon reconstitution in cold water. Concurrently, some spray-drying parameters were tested, in particular different outlet air temperatures were applied (55, 70 and 85 °C).

2. Material and methods

2.1. Material

Micellar casein powder (Promilk 872 B) and whey protein powder (Promilk 752 FB) were purchased from Ingredia IDI (Arras, France). Chymosin (Chymax Plus) was provided by CHR Hansen (Hørsholm, Denmark).

2.2. Preparation of proteins and chymosin solutions

Micellar casein and whey protein solutions were prepared separately by rehydrating powders in distilled water at 12.5% (w/w) dry extract. Rehydration was with an overhead stirrer equipped with a spiral stirrer (IKA, Staufen, Germany) at a speed of 1000 rpm for 2 h at room temperature (20 °C), then overnight (4 °C). After rehydration, whey proteins were denatured by heating the solution at 78 °C during 10 min (Petit et al., 2011) and the solution was cooled at 4 °C. The chymosin solution was prepared by diluting ten times the initial solution (200 IMCU mL^{-1}) in distilled water.

2.3. Preparation of bacterial suspension

The strain used in this study was *L. rhamnosus* GG (ATCC 53103). The growth of *L. rhamnosus* GG was performed in a laboratory-scale reactor. Bacterial stocks used for the inoculation were stored at -20 °C in MRS broth with 20% (v/v) glycerol. A pre-culture was prepared by inoculating *L. rhamnosus* GG in 200 mL of MRS broth at 37 °C for 15 h. The bioreactor containing 1 L of fresh medium was inoculated with the whole pre-culture. Growth was performed at 37 °C under agitation at 300 rpm and pH was adjusted at 6.8 with the addition of 6 M NaOH. Cells concentration was determined by following the absorbance at 660 nm. Culture was stopped at the beginning of the stationary phase and bacterial cells were harvested from the broth by gentle centrifugation (3000 *g*, 10 min). The pellet was then lyophilized during 72 h and stored at 4 °C before use.

2.4. Production of microparticles by spray-drying

The concentrate was prepared by mixing micellar caseins and denatured whey proteins solutions in a ratio of 90/10 (v/v), respectively. Before spray-drying, the chymosin solution was added to the concentrate at a final chymosin concentration of 12 IMCU g^{-1} of proteins. The protein concentrate in presence of chymosin was left for 30 min at a temperature of 8 °C to allow caseinomacropeptide cleavage through the action of chymosin and to avoid casein coagulation (Burgain et al., 2013). After the enzymatic cleavage step, lyophilized *L. rhamnosus* GG was mixed with the concentrate at approximatively 8.0 $\log_{10}$ CFU g^{-1} before spray-drying. After incubation, the solution containing *L. rhamnosus* GG was spray-dried using a pilot-scale spray drier MicraSpray 150 (Anhydro, Soeborg, Danemark). A peristaltic pump was used to deliver the liquid through the bi-fluid nozzle into the spray-drying chamber with a feed flow-rate of 87 mL min^{-1} and a nozzle pressure of 1 bar. In this study, different theoretical outlet air temperatures were tested: 85, 70 and 55 °C. The real (measured) inlet and outlet temperatures were collected in Table S1. The spray-dried microparticles were stored in plastic vessel at 4 °C before use. Two independent productions were realized. A thermo-humidity sensor was placed at the output of the cyclone to measure the relative humidity and the temperature of the humid air (Table S1).

2.5. Survival of L. rhamnosus GG after spray-drying

To evaluate the survival of *L. rhamnosus* GG during encapsulation, cell counts were determined before and after spray-drying. Cell counts were obtained by determining the number of CFU in 1 mL concentrate before spray-drying and in 1 g spray-dried powder. For this purpose, 1 mL (concentrate) or 1 g (powder) sample was introduced in 9 mL of tryptone salt broth and the solution was mixed during 2 min using a vortex homogenizer. Sample was serially diluted in tryptone salt broth and plated on MRS agar. After 48 h incubation at 37 °C, cell counts were determined and expressed as CFU g^{-1}. The concentrate density was measured to be able to translate the 1 mL of concentrate in grams. For this, a known volume of concentrate was weighed. The experiment was realized