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Double use of concentrated sweet whey for growth and spray drying of probiotics: Towards maximal viability in pilot scale spray dryer

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ABSTRACT

Spray-drying is expected to be a cost-efficient way to produce probiotic powders. Indeed, a novel simplified process was recently reported, using concentrated sweet whey (30 wt %) as a sole medium for both growth and spray drying of probiotics. The feasibility of scaling up this process was validated in the present work with a semi industrial pilot scale spray dryer. A multi-stage mild-conditions drying process, coupling spray-drying with belt drying and fluid-bed drying, was also applied in this work, in which the final probiotic survival was improved to approximately 100% (>10^9 CFU g^{-1}). The change of probiotic viability in the powders was monitored during a 6-month storage, which indicated that storage temperature and moisture content of powders play crucial roles in the stability of probiotic powders. Moreover, spray-drying afforded a strain-dependent enhancement of bacterial tolerance in simulated intestinal fluid, in comparison with fresh cultures.

1. Introduction

Probiotics are live microorganisms which, when administered in adequate amounts, confer a beneficial health effect on the host (FAO/WHO, 2001). Probiotic products are highly demanded because of the increasing market in the current era, in which probiotic powders are preferred by industries, due to their storage stability and convenience for both transport and incorporation within various foods. The specific functionality of probiotic strains is suggested to depend on the adequate ingested dose (Johansson et al., 2015; Zhu et al., 2014). Hence, high viability of probiotics is desired when developing probiotic powders. To preserve probiotic viability during production, long-term storage and digestion thus become a challenge in both scientific and technical perspectives (Tripathi and Giri, 2014).

Freeze drying is the main method currently employed to produce dried probiotics. However, considering the high production cost and low productivity of freeze drying, spray drying is expected to be an alternative method for production of probiotic powders at an industrial scale: indeed, the specific energy consumption of spray drying is more than 10 times lower than that of freeze drying (Schuck et al., 2013). However, the harsh spray drying conditions, in particular high temperature exposure at the last stage of drying, limit the applicability of spray drying in probiotic production (Fu and Chen, 2011; Peighambardoust et al., 2011). Extensive studies thus have been carried out to find the strategy in order to improve probiotic viability during spray drying. For example, pretreatment of bacteria with sub-lethal doses of stress has been reported to be an effective route to induce bacterial tolerance against spray drying (Desmond et al., 2001). In addition, using a protective matrix as a drying medium represents another strategy to protect bacteria from spray drying (De Castro-Cislaghi et al., 2012; Perdana et al., 2014). Finally, the drying conditions can also be moderated through technical innovation or process optimization, decreasing drying temperature by multi-stage drying process for instance (Schuck et al., 2013). Despite these advances, it is worth noting that these strategies were mostly applied at laboratory-scale, while their validation at pilot or industrial scale spray drying is rarely reported.

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In a recent work, a novel process used for spray drying of probiotics was developed by double-use of concentrated sweet whey (30 wt%) as a sole medium for both growth and spray drying of probiotic *Lactobacillus casei* BL23 and *Propionibacterium freudenreichii* ITG P20 (Huang et al., 2016a). It was shown that the hypertonic stress in this concentrated sweet whey led to overexpression of key stress proteins, accumulation of intracellular storage molecules, of compatible solutes, enhanced multistress tolerance acquisition, as well as enhanced survival upon spray drying (Huang et al., 2016b). The high solid content of the feed (i.e. bacterial culture) furthermore facilitated the spray drying process, making it possible to lower the drying temperature. In the current paper, the feasibility of scaling up this process is investigated from lab scale to semi industrial pilot scale. Besides, a previously reported multi-stage spray drying process is applied to further improve the probiotic viability after drying (Schuck et al., 2013). The storage stability of probiotic powders, as well as their protection towards digestion, are also investigated.

2. Materials and methods

2.1. Strains and pre-culture

Lactobacillus casei BL23 was provided by UMR1219 MICALIS, (INRA-AgroParisTech, Jouy-En-Josas, France) and *Propionibacterium freudenreichii* ITG P20 was maintained and pre-cultured by the CIRM-BIA Biological Resource Center (Centre International de Ressources Microbiennes-Bactéries d'Intérêt Alimentaire, INRA, Rennes, France). *L. casei* was activated by inoculation (1% inoculum size) in MRS Broth and static cultivation at 37 °C for 16 h. *P. freudenreichii* was activated (1% inoculum size) in YEL broth and cultivated statically at 30 °C for 50 h.

2.2. Fermentation with sweet whey

Sweet whey medium with 30 wt% total solid content was prepared by rehydration of sweet whey powder (Lactalis ingredients, Mayenne, France) in deionized water.

For preparation of starter culture, the 5 L sweet whey was autoclaved at 100 °C for 30 min before inoculation of *L. casei* or *P. freudenreichii*. The inoculation (1 v/v% inoculum size) was made from the above-mentioned pre-culture of *L. casei* in MRS broth or of *P. freudenreichii* in YEL broth. The inoculated sweet whey culture of *L. casei* was incubated statically at 37 °C for 30 h, and *P. freudenreichii* at 30 °C for 72 h. The obtained culture was used as starter for further fermentation.

Sweet whey medium for fermentation at the semi industrial pilot scale was prepared in a steel tank (Goavec, Alençon, France) by rehydrating 150 kg sweet whey powders in 350 kg water to obtain 30 wt% total solid content. The medium was then pumped through a scraped surface heat exchanger (HRS Heat Exchangers, France) for heat treatment. The heating temperature and the residence time of sweet whey medium within the heat exchanger were 120 °C and 1 min, respectively. The heated sweet whey medium was then transferred into a 500 L bio-reactor (Goavec, Alençon, France). The pipes and the bio-reactor were previously treated by steam. The above-mentioned 5 L starter culture was inoculated after the temperature of sweet whey had been cooled down to the setting growth temperature (i.e. 37 °C for *L. casei* and 30 °C *P. freudenreichii*). The sweet whey with *L. casei* was fermented statically at 37 °C for 48 h, and *P. freudenreichii* at 30 °C for 96 h.

2.3. Spray drying

Before spray drying, the sweet whey probiotic culture was agitated moderately for 20 min. Three processes of spray drying were performed for each probiotic strain (Fig. 1). For the lab-scale spray drying (Fig. 1a), 1 L sweet whey culture was pumped to a Mobile Minor™ spray dryer (GEA Niro A/S, Denmark). A two-fluid spray nozzle with an orifice diameter of 0.8 mm was used. The evaporation rate of this dryer was approximately 3 kg h^{-1}. The inlet air temperature was at 140 ± 1 °C, and the outlet air temperature 60 ± 3 °C.

The one-stage semi industrial pilot scale spray drying (Fig. 1b) was performed in the Bionov spray dryer pilot workshop (Niro Atomizer, GEA, Saint Quentin en Yvelines, France) based in Rennes (France). A pressure nozzle with an orifice diameter of 0.73 mm was used. The evaporation capacity was approximately 80 kg h^{-1}. The drying temperatures were set to the same values as in minor dryer, namely inlet air temperature at 140 ± 5 °C and outlet air temperature at 60 ± 3 °C. A belt (GEA, Saint Quentin en Yvelines, France) and a vibro-fluidizer (GEA, Saint Quentin en Yvelines, France) were used following the spray drying step (Fig. 1b), but respectively for conveying (turn-off status) and powder cooling purposes in this configuration.

The multi-stage semi industrial pilot scale spray drying (Fig. 1c) was also performed using the Bionov spray dryer pilot workshop, as described before (Schuck et al., 2013). Briefly, the inlet air temperature of spray drying was decreased to 127 ± 3 °C, and the outlet air temperature 47 ± 2 °C. After spray drying, the partially dried powder was delivered through the belt dryer at ambient temperature for crystallization purpose. The residence time of powders on belt was approximately 5 min. Fluid-bed drying was then carried out in a vibro-fluidizer (VF) with inlet temperature at 80 ± 2 °C and outlet temperature at 40 ± 2 °C.

2.4. Analysis of physical and chemical properties

For liquid samples, the pH value was measured with a pH-meter (Ecolab, Issy-lès-Moulineaux, France). Viscosity measurements were performed using an AR 2000 rheometer (TA instruments, Guyancourt, France) equipped with coaxial cylindrical geometry (stator inner radius: 25 mm; rotor outer radius: 23 mm; immersed cylinder height: 30 mm; bottom cap: 4000 μm). Apparent viscosity was determined at 20 °C using the Herschel–Bulkley model at a shear rate of 1 s^{-1}. The water content of liquid or powder samples was measured according to the method described by Schuck et al. (2012): the sample (respectively 5 g for liquid samples and 1 g for powder samples) was mixed with 25 g pre-dried sand and then dried at 105 °C for 7 h (liquid samples) or 5 h (powder samples). The water activity of powders was determined at 25 °C using an a_w-meter (Novasina, a_w-center 92T0003) immediately after drying and cooling.

2.5. Storage of powders

The powders were collected and aliquoted in PA/PE plastic vacuum bags (La Bovida, France). The bags were then sealed in the presence of air or under vacuum conditions (Britek, France) respectively. These powders were stored at the controlled temperature of 4 °C and 25 °C and kept away from light.

2.6. In vitro simulated digestion

The effect of spray drying on the probiotic tolerance against digestion stress was investigated in the *in vitro* simulated digestion experiment. The powders obtained from multi-stage drying process (Fig. 1c; powder 3) were used to compare with the fresh