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Short communication

Microencapsulation of *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and *Propionibacterium jensenii* 702 by spray drying in goat's milk

C. Senaka Ranadheera[a,b,*], Craig A. Evans[a], Michelle C. Adams[a], Surinder K. Baines[c]

[a] School of Environmental and Life Sciences, University of Newcastle, New South Wales 2308, Australia
[b] Department of Animal & Food Sciences, Faculty of Agriculture, Rajarata University of Sri Lanka, Anuradhapura, Sri Lanka
[c] School of Health Sciences, University of Newcastle, New South Wales 2308, Australia

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ABSTRACT

A mixture of probiotic *Lactobacillus acidophilus* LA-5, *Bifidobacterium animalis* subsp. *lactis* BB-12 and novel potential probiotic *Propionibacterium jensenii* 702 was resuspended in reconstituted (20% w/v) goat's milk, spray dried in a mini spray dryer (inlet temperature = 195 °C and outlet temperature = 85 °C) and the spray dried powder was stored in air tight glass jars at 4 °C and 30 °C for 24 weeks. Powder quality and probiotic viability after spray drying and subsequent storage were measured. Spray drying probiotics in reconstituted goat's milk resulted in a significant reduction in the viability of all three probiotics. However, all three probiotics were able to maintain satisfactory viability levels (10^6–10^8 cfu/g) after spray drying. While storage temperature did not appear to have a significant effect on moisture content, the viability of all three strains declined dramatically when stored at 30 °C but lactobacilli and propionibacteria remained virtually unaffected under storage at 4 °C, satisfying recommendations regarding the level of viable cells in probiotic foods.

1. Introduction

Spray drying is one of the common methods used to prepare food particles which are dry, stable and occupy small volumes (Gardiner et al., 2000; Lian et al., 2002), and can be considered the most widely used microencapsulation technique in the food industry (Desai and Hyun-Jin, 2005). Spray drying has been identified as a processing technique which improves the survival of probiotics in food during processing and storage with some additional benefits such as protection of probiotics against subsequent exposure to the harsh conditions of the gastrointestinal tract (Kent and Doherty, 2014), because the process encases the bacterial cells in an outer protective coat. Further, encapsulated probiotics are protected from bacteriophage and harsh conditions such as freeze storage (Anal and Singh, 2007). Microencapsulation has also potential in reducing post fermentation acidification and possible negative sensory effects of probiotic food products (Sohail et al., 2012).

Although spray drying may have positive effects on probiotic survival, several factors may contribute to a reduction in the survival rate of probiotics during spray drying and subsequent storage, including airflow

* Corresponding author at: School of Environmental and Life Sciences, University of Newcastle, New South Wales 2308, Australia.
Tel.: +61 416804607; fax: +61 2 49216925.
E-mail addresses: senakar@email.com, senaka.ranadheera@uon.edu.au (C.S. Ranadheera).

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configuration, dehydration, spray drying temperature conditions, concentration of the spray dried suspension, concentration of the probiotics in the suspension, the carrier materials used in the process, species/strain specific factors, storage temperature and packaging (Ho, 2008). Higher probiotic survival rates have been previously obtained at lower storage temperatures (4–8 °C) compared to higher storage temperatures (15–30 °C) (Gardiner et al., 2000; Teixeira et al., 1995). However, as a dried product, storage at room temperature may be desirable for spray dried probiotic powders, especially in commercial applications due to the higher operational costs associated with refrigerated storage, difficulties in transport and distribution as well as limited availability of cold storage facilities in certain areas of the world.

Spray drying has been extensively used in the dairy industry, primarily to maintain starter cultures. Generally microencapsulated probiotics have a tendency to survive better in dairy foods compared to free form of the same strains. Many authors have utilized cow's milk as a carrier agent in spray drying probiotics (Chavez and Ledeboer, 2007; Fritzen-Freire et al., 2012; Kent and Doherty, 2014). Although goat's milk can be successfully utilized in spray drying (Reddy et al., 2014), to the best of the author's knowledge goat's milk has not been utilized as a carrier solution/suspension in spray drying probiotics. The use of goat's milk as a carrier material in the spray drying of probiotics may provide several advantages. It can be directly used as a probiotic food or can be used as inoculum for probiotic goat's milk products without any risk of contamination of other carrier materials such as cow's milk. Further many authors have used probiotic strains belong to genera lactobacilli (dos Santos et al., 2014; Golowczyc et al., 2011) and/or bifidobacteria (Fritzen-Freire et al., 2012, 2013) for spray drying microencapsulation, however, other genera such as *Propionibacterium* have gained less attention up to date. This study aimed to evaluate the efficacy of spray drying of the novel potential probiotic *Propionibacterium jensenii* 702 together with *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 in goat's milk and to examine their storage stability.

2. Materials and methods

2.1. Spray drying process and microbiological analyses

L. acidophilus LA-5 and *B. animalis* subsp. *lactis* BB-12 (CHR Hansen Pty Ltd., Bayswater, VIC, Australia) were obtained as freeze dried cultures and novel potential probiotic *P. jensenii* 702 was obtained from a stock culture maintained at the University of Newcastle, Australia. *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 were grown in MRS broth and RC medium (Oxoid Australia Ltd., Adelaide, Australia) respectively (anaerobic incubation, 37 °C for 24 h) while *P. jensenii* 702 was anaerobically incubated at 30 °C in SL broth for 72 h.

Since it has been reported that the bacteria in their lag and exponential/log growth phases are more susceptible to heat than bacteria in their stationary phase (Corcoran et al., 2004), in the present study probiotic cells were harvested in their stationary phase for spray drying. Bacterial cells were harvested from the broths by centrifugation (2500 × *g*, 10 min, 4 °C) (Eppendorf centrifuge 5810R, Germany), washed three times with 0.1% sterile saline solution and resuspended (inoculation level 10^8–10^9 cfu/ml each bacteria) in reconstituted (20% w/v) goat's milk (Healtheries of New Zealand Ltd., Auckland, New Zealand), heat treated at 85 °C for 30 min before being cooled to the inoculation temperature (37 °C). Immediately after inoculation the probiotic bacterial suspensions in reconstituted milk were processed using a laboratory scale spray dryer (Buchi mini spray dryer B-290, Flawil, Switzerland). Samples were processed at a constant feed rate (pump feed rate 40%), air spray flow of 600 l/h, 100% aspirator setting and at 195 °C air inlet temperature. The resultant outlet air temperature was maintained at 85 ± 2 °C. These spray drying conditions were determined by series of preliminary experiments. The spray dried powder was stored in air tight glass jars at 4 and 30 °C for 24 weeks.

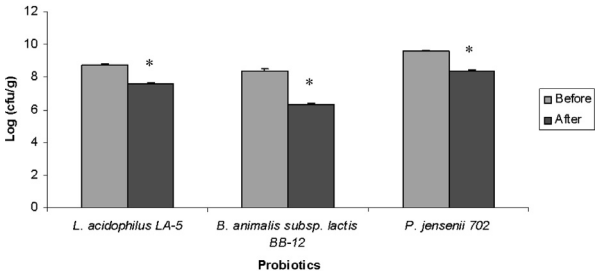

Fig. 1. Viable probiotic cell counts before and after spray drying. Asterisk (*) indicates a significant difference between corresponding before and after cell counts ($p < 0.05$) ($n = 4$).

Spray dried powder samples were used to enumerate probiotics immediately after spray drying as well as during storage. Serial dilution and spread plating were performed to determine total viable probiotic counts. MRS–sorbitol agar was used for the selective enumeration of *L. acidophilus* LA-5 while MRS–NNLP agar was prepared for the selective enumeration of *B. animalis* subsp. *lactis* BB-12 (37 °C for 72 h under anaerobic condition). SLA was used for the selective enumeration of *P. jensenii* 702 (30 °C, 5–7 days, anaerobic incubation).

2.2. The moisture content

Moisture content of the spray dried powder was also determined at the time of production and 24 weeks after production by oven-drying samples to constant weight at 105 ± 1 °C in pre-dried porcelain crucibles.

2.3. Scanning electron microscopy

Samples of spray dried powder were spread thinly onto a double-sided carbon adhesive disc, anchored to the electron microscopy stub, coated with a 20 nm layer of gold particles and then examined under a scanning electron microscope (Philips XL30, Philips, Eindhoven, The Netherlands).

2.4. Statistical analysis

Data analyses were performed using SPSS/PASW statistical software version 17 (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered statistically significant for all analyses.

3. Results and discussion

3.1. Quality of the spray dried powder and probiotic viability during spray drying

Both *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12 required 24 h of anaerobic incubation at 37 °C in MRS broth and RC medium respectively to enter their stationary phases while *P. jensenii* 702 required 72 h of anaerobic incubation at 30 °C in SL broth. The maximum population was found to be ~4.2×10^8 cfu/ml for both *L. acidophilus* LA-5 and *B. animalis* subsp. *lactis* BB-12, and ~10^9 cfu/ml for *P. jensenii* 702 at their stationary phases.

Spray drying caused a significant viability loss in all three probiotics (Fig. 1), probably due to the higher temperatures involved in the process. Reduction in cell viability during spray drying was most likely due to heat inactivation (To and Etzel, 1997) and has previously been reported