Received: 24 January 2022 | Revised: 18 May 2022 | Accepted: 22 June 2022

DOI: 10.1111/jfpp.16880

Journal of Food Processing and Preservation | Institute of Food Science + Technology | ifst | WILEY

ORIGINAL ARTICLE

Microencapsulation of *Limosilactobacillus reuteri* DPC16 by spray drying using different encapsulation wall materials

Fang Wang | **Anthony N. Mutukumira**

School of Food and Advanced Technology, Massey University, Auckland, New Zealand

Correspondence
Anthony N. Mutukumira, School of Food and Advanced Technology, Massey University, Auckland, New Zealand.
Email: a.n.mutukumira@massey.ac.nz

Funding information
Massey University

Abstract

Most probiotics are delivered in liquid media which tends to limit their shelf life thus, they are not convenient for the modern lifestyles. In this study, *Limosilactobacillus reuteri* DPC16 was encapsulated in 10% reconstituted skim milk (RSM), 10% gum Arabic, 10% maltodextrin, and a mixed wall material (2.5% whey protein isolate/2.5% gum Arabic/2.5% inulin/2.5% sucrose), (w/w). The mixture was then spray-dried at 160°C/80°C and/or 180°C/100°C inlet/outlet temperatures. The spray-dried DPC16 microcapsules were characterized for viable cells of the probiotic, a_w, and morphology. Results showed that at the inlet/outlet temperatures of 160°C/80°C, RSM as an encapsulation wall material had the highest cell counts (98.06% ± 0.86%) with 0.196 ± 0.010 a_w. Most of the powder particles (RSM) were spherical with dented surfaces. At 180°C/100°C, about 84% DPC16 cells survived in RSM capsules. Thus, RSM showed good potential as an encapsulating wall material to maintain high DPC16 cell viability during spray drying.

Novelty impact statement: The encapsulation of *Limosilactobacillus reuteri* DPC16 using reconstituted skim milk (RSM) by spray drying at elevated inlet/outlet temperatures had lower encapsulation efficiency (EE), water activity, and smaller particle size of the microcapsules compared to RSM microcapsules spray-dried at low temperatures. Mixed wall material comprising gum Arabic, whey protein isolate, inulin, and sucrose produced microcapsules with relatively high EE, low water activity, and some microcapsules with smoother surfaces which could improve powder solubility.

1 | INTRODUCTION

The concept of functional food has now moved to gastrointestinal health and its relationship with the gut bacteria. This novel concept might be caused by the pervasiveness of gastrointestinal diseases as diet is an important factor that affects the activities of indigenous gut microbiota (Anandharamakrishnan, 2015; Pimentel et al., 2021). The demand for supplementary functional health products for food has contributed to the research and development of probiotics.

Probiotics originated from the Greek word "pro bios", whose meaning is "for life" (Pimentel et al., 2021) is a dietary supplement. A bacterium may be described as a probiotic only when it can be proven to be alive when it is consumed in an adequate amount to confer health benefits (FAO/WHO, 2002; Pimentel et al., 2021; Sahin et al., 2007). For a probiotic functional food, the product needs to contain at least 10^6 live cells per gram or milliliter at the time of consumption (FAO/WHO, 2002; Ishibashi, 1993; Krasaekoopt et al., 2003). However, during processing and storage, products may be exposed to moisture, oxygen, shear, light, and heat that

contribute to increased cell death. All these challenges suggest that the use and application of probiotics in food are still very limited (Kailasapathy, 2002). Therefore, there is a need to develop innovative methods to protect probiotic microorganisms during processing, storage, and handling.

Several strategies have been proposed to enhance the viabilities of probiotics such as strain selection, strain adaption in the gastrointestinal tract (GIT) or food matrix, packaging system, protection from intrinsic and extrinsic factors, and addition of probiotic-promoting (prebiotics) compounds (Terpou et al., 2019). Several technologies can be used to achieve the protection of probiotic cells during processing and storage including conventional freeze drying and microencapsulation. The latter (microencapsulation) has been proven superior on the stability of cells even in the GIT using different materials and methods, and has become the main modern solution to preserve probiotic viability (Călinoiu et al., 2019). Microencapsulation is a technology which can enclose solids, liquids as well as gaseous components thereby protecting the contents (Champagne & Fustier, 2007; Mutukumira et al., 2015). In this process, individual particles of active components are enclosed in a shell by coating with a continuous outer layer to protect the inner components.

Several processes have been used for microencapsulation, such as spray drying, freeze drying, extrusion, emulsion, and fluid-bed drying (Burgain et al., 2011; Champagne & Fustier, 2007; Thantsha et al., 2009). Spray drying is recommended for application in industry because it is relatively economical, easy to scale up (Prüsse et al., 2008), does not need the use of unsafe solvents, and the powder produced is stable at ambient temperature. However, spray drying tends to damage cells due to the high temperatures used during drying. Thus, proper biopolymers have to be used to protect the cells. Natural gums (gum Arabic, alginates, carrageenan, etc.), proteins (milk or whey protein, gelatin), and carbohydrates (maltodextrins with different dextrose equivalent) (Gharsallaoui et al., 2007) have all been investigated for microencapsulation by spray drying.

For spray-dried microcapsules containing probiotics, encapsulation efficiency is of paramount importance as it reflects the ability of the wall material to protect cells from high temperatures during spray drying (Rajam & Anandharamakrishnan, 2015). It is well-known that water activity significantly affects the survival of microorganisms (Jay et al., 2008), whereas particle surface affects function of powder properties such as rehydration, caking, flowability, and stickiness (Gaiani et al., 2013). According to our knowledge, there is no published information on the microencapsulation of the probiotic culture DPC16 by spray drying. Therefore, the aim of this study was to select a suitable wall material which gives high encapsulation efficiency (EE) with low a_w and optimal surface appearance to encapsulate *Limosilactobacillus reuteri* DPC16 by spray drying.

2 | MATERIALS AND METHODS

All the experiments were repeated twice, and duplicate samples were analyzed for each treatment. The chemicals used in the experiments were of reagent grade or higher. All microbiological media used were sterilized at 121°C for 15 min in an autoclave (AMA040, Astelle Scientific, Sidcup, UK) according to the instructions of the supplier. Glassware and other equipment were sterilized by dry heat at 105°C for at least 3 h using the hot air oven (Soil drying oven, Unitemp, Queensland, Australia).

2.1 | Description of microencapsulation materials

The wall materials used for the encapsulation of DPC16 were selected based on previous studies (Anandharamakrishnan, 2015; Fritzen-Freire et al., 2012; Gul, 2017; Ivanovska et al., 2015; Lakkis, 2007; Liao et al., 2017; Ying et al., 2012). The materials used were as follows: gum Arabic (Caldic, Rotterdam, Netherland); maltodextrin (Interchem, Auckland, New Zealand); commercial Anchor™ skim milk powder containing 0.62% milkfat (Fonterra, Auckland, New Zealand); inulin (Caldic, Rotterdam, Netherland); whey protein isolate (Caldic, Rotterdam, Netherland); and, sucrose (Crescendo, Auckland, New Zealand).

The wall materials used in this study were 10% reconstituted skim milk (RSM) (w/w), 10% gum Arabic (w/w), 10% maltodextrin (w/w), and the mixed wall material (MWM) containing 2.5% whey protein isolate (w/w), 2.5% gum Arabic (w/w), 2.5% inulin (w/w), and 2.5% sucrose (w/w). The inlet/outlet temperatures used were 160°C/80°C and/or 180°C/100°C (Anandharamakrishnan, 2015; Fritzen-Freire et al., 2012; Gul, 2017; Ivanovska et al., 2015; Lakkis, 2007; Liao et al., 2017; Ying et al., 2012). The experimental setup of the conditions for the microencapsulation of DPC16 by spray drying is shown in Table 1.

2.2 | Preparation of liquid suspension containing DPC16 (feed solution) for spray drying

The frozen *L. reuteri* DPC16 probiotic culture was supplied by Bioactive Research New Zealand Ltd (Auckland, New Zealand). DPC16 cells were preserved in cryo-vials (Microbank™, Pro-Lab Diagnostics, Birkenhead, UK). One bead was withdrawn from the

TABLE 1 Treatment conditions for the microencapsulation of *Limosilactobacillus reuteri* DPC16 by spray drying

Experiment #	Wall material	Concentration (%, w/w)	T_{inlet} (°C)	T_{outlet} (°C)
1	RSM	10	160	80
2	gum Arabic	10	160	80
3	maltodextrin	10	160	80
4	MWM	10	160	80
5	RSM	10	180	100

Abbreviations: MWM = mixed wall material comprising 2.5% (w/w) whey protein isolate/2.5% gum Arabic (w/w)/2.5% inulin (w/w)/2.5% sucrose (w/w); RSM = reconstituted skim milk; T_{inlet} = inlet temperature; T_{outlet} = outlet temperature.

frozen vial and inoculated into 9 ml of MRS broth (Merck, Darmstadt, Germany), followed by incubation for 18 h at 37°C anaerobically. After incubation, 0.4 ml were inoculated into 40 ml of fresh MRS broth and then incubated anaerobically for another 18 h at 37°C for sub-culturing. The anaerobic environment was created by an anaerobic sachet (Thermo Scientific™ Oxoid AnaeroGen 2.5 L Aachets, Thermo Fisher Scientific, Waltham, US) placed in an air-tight jar (Thermo Scientific™ Oxoid 2.5 L jar, Thermo Fisher Scientific, Waltham, US).

DPC16 cells were harvested by centrifugation (Sigma 6–16 KS, Sigma Laboratory Centrifuge, Osterode am Harz, Germany) at 3200 × g for 10 min at 4°C. The cell pellets were washed twice by suspending in 0.1% peptone water (w/w) (Merck, Darmstadt, Germany) and swirled gently before centrifugation. The supernatant was discarded, then the optical density (595 nm) of the cell suspension was adjusted using peptone water to an absorbance of 0.5 (giving ≈10^8 CFU/ml) in a spectrophotometer (Novaspec III, Amersham Biosciences, Amersham, UK). About 10 times dilution (compared to the volume of the growth medium, MRS broth) was required to achieve an absorbance of 0.5 using peptone water. Thus, approximately 400 ml of peptone was needed. Then the cell suspension was centrifuged at 3200 × g for 10 min and the supernatant discarded, leaving the cells attached to the wall of the centrifuge tube to be mixed with the wall material solution. To maintain consistency of the initial cell numbers for encapsulation by spray drying, cells were mixed with 400 ml of each wall material colloid.

The wall material colloids were prepared by dissolving each wall material in distilled water to produce a solid–liquid ratio of 10% (w/w). The prepared colloids were kept in ice-bath (≈ 8°C) with continuous agitation (300 rpm) using an overhead stirrer (RW 20.n, IKA laboratory technology, Rawang, Malaysia) for at least 30 min to remove any lumps.

After the temperature of the wall material colloids had stabilized at ≈ 8°C in the ice-bath, the sediment of cells in the centrifuge tube (250 ml) to which cells had attached was mixed with the wall material colloid. Cells attached onto the wall of the centrifuge tube were dislodged by a pipette and gentle swirling. After mixing, the mixture in the centrifuge tube was transferred into the beaker containing the rest of the wall material solution, which constituted the final feed solution. The feed solution was immediately pumped into the dryer (Figure 1) (after the spray dryer had stabilized at the desired levels of inlet/outlet temperatures) with continuous stirring at 300 rpm using an overhead stirrer.

2.3 | The spray-drying process

The Saurin SL-10 pilot scale spray dryer (Saurin Enterprises Pty, Port Melbourne, Australia) (Figure 1) was used to spray dry a liquid suspension containing viable cells of the DPC16 encapsulated in each respective wall material.

During the spray-drying process, the feed solution was kept in the ice-bath (≈ 8°C) to minimize the growth of the cells. Samples for the determination of the cell concentration in the feed solutions were collected in duplicate (from the feed solution) at two time points: before pumping the feed solution into the spray dryer and just before the end of the feeding process.

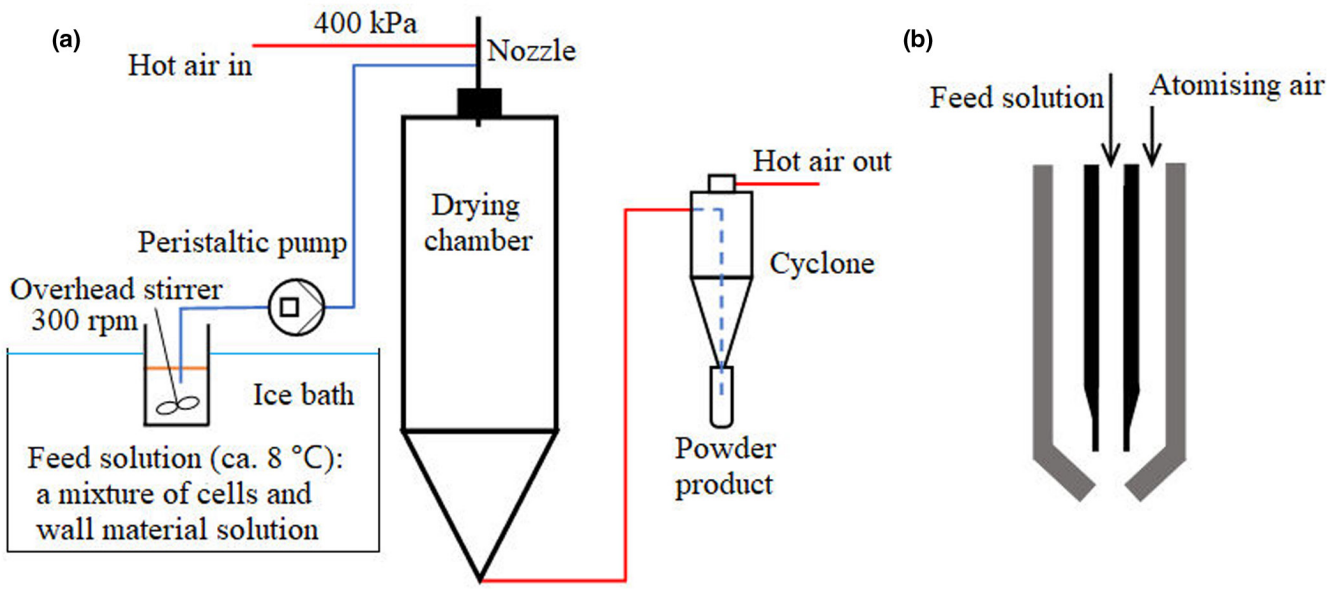

FIGURE 1 (a) Schematic diagram of the single effect spray-dryer, (b) cross-section of a pneumatic (twin fluid) nozzle. Figure 1 was drawn using Microsoft Paint (Microsoft Office 2013, Microsoft, US), based on the Saurin SL-10 operating manual (SL- 10, Saurin, Australia)

2.4 | Packaging of the powder product after spray drying

Following spray drying, the dry powder (approximately 20 g) was collected and packed in aluminum foil bags (PET/AL/LDPE) (ALFW5-18, PBAG, China) and heat-sealed by a foot-operated heat sealer (Hardy Packaging Ltd., New Plymouth Central, New Zealand).

2.5 | Determination of encapsulation efficiency

The encapsulation efficiency was calculated using Equation (1):

$$\%\,\text{Encapsulation Efficiency} = \frac{N}{N_0} \times 100\,\% \quad (1)$$

Where N_0 is the log number of viable cells in CFU/g of a wall material on a dry basis; N is the log number of viable cells in CFU/g of spray-dried microcapsules (Rajam & Anandharamakrishnan, 2015).

Cell concentration was analyzed using the pour-plate method within 3 h after spray drying. To analyze the cell concentration of feed solution, suitable serial dilutions were prepared and plated in MRS agar (Oxoid, Thermo Fisher Scientific, Waltham, USA) and incubated anaerobically at 37°C for 48 h. For analysis of the cell concentration of the spray-dried powder, 10 g were dissolved in 0.1% (w/w) peptone water to make a 10% (w/w) solution. Suitable serial dilutions were then plated as described earlier in this section.

2.6 | Determination of the water activity of DPC16 microcaspsule powders

The water activity of the spray-dried powders was measured using the AquaLab Series 3 water activity meter following the supplier's instructions (Decagon Devices Inc., Pullman, USA). The water activity (25°C) of the DPC16 microcapsule powder samples (2 g) was measured in duplicate immediately after production using the water activity meter. The meter was calibrated at 25°C using the supplied commercial standard samples prior to use. In this paper, the term "particle" is synonymous with the term "microcapsule" or "capsule."

2.7 | Determination of the surface appearance of the DPC16 microcapsule powder

The surface appearance of the DPC16 microcapsule powder was determined based on the scanned electron micrograph (SEM) captured by the FEI Electron Optics, Quanta 200 (FEI Company, Hillsboro, USA). The method used to determine surface appearance was based on the report by Tan and Balasubramanian (2017) with minor modifications.

The (probiotic) powder samples for the SEM imaging were prepared in a temperature-controlled room (22°C). The powders were sprinkled on a double-sided tape (Deskwise, Auckland, New Zealand) on the supplied aluminum sample holder and excess particles of the powder were removed using a rubber suction bulb (60 ml Laboratory Tool, Burry Life Science, UK). The sample was then examined under the scanning electron microscope unit using an accelerating voltage of 10.00 kV. Digital images were captured at ×2000 and ×16,000 magnifications. The surface smoothness of the microcapsules spray-dried at different conditions was observed and compared using the SEM images magnified at ×2000 and ×16,000.

2.8 | Data analysis

The data of EE and water activity were analyzed by the Analysis of Variance (ANOVA) using the General Linear Model (GLM) to determine the effects of wall material, batch, and inlet/outlet temperatures on the EE of each wall material and water activity of the DPC16 microcapsule powder ($p < .05$). Significant differences between means of data were separated using Tukey's Pairwise comparison test (Maciel et al., 2014).

3 | RESULTS

3.1 | Encapsulation efficiency

At the inlet/outlet temperatures of 180°C/100°C, about 84% of DPC16 cells survived when they were encapsulated in 10% RSM (w/w) (data not shown). However, at the inlet/outlet temperatures of 160°C/80°C, the EE of all wall materials was above 90.00% (Table 2). The RSM had the highest EE (98.06 ± 0.86%), followed by the powder made from the MWM with an EE of 93.97 ± 1.49%. Maltodextrin and gum Arabic had lower EE at 92.50 ± 0.37% and 90.63 ± 3.08%, respectively. Results showed that both the drying temperature and type of the wall material affected the EE of a wall material ($p < .05$).

3.2 | Water activity

Table 2 shows the water activity of DPC16 microcapsules made from different wall materials and spray-dried at 160°C/80°C. The results show that: gum Arabic (0.170 ± 0.005) < MWM (0.196 ± 0.010) < maltodextrin (0.237 ± 0.010) < RSM (0.284 ± 0.005). Water activity of the DPC16 microcapsules made from RSM and spray-dried at 180°C/100°C was 0.200 ± 0.004 (data not shown). Results show that both the encapsulation wall materials and inlet/outlet temperatures had a significant effect on water activity of the spray-dried DPC16 microcapsules ($p < .05$).

3.3 | Surface appearance

As shown in Figure 2-1a,b, microcapsules made from RSM and spray-dried at 160°C/80°C were irregular or spherical in shape with

TABLE 2 The encapsulation efficiency (EE) and water activity of spray-dried *Limosilactobacillus reuteri* DPC16 microcapsules

	Encapsulation wall material			
Parameter	**Maltodextrin**	**Gum Arabic**	**RSM**	**MWM**
EE	92.50 ±0.37%	90.63 ±3.08%	98.06 ±0.86%	93.97 ±1.49%
Water activity	0.2365 ±0.0097	0.1695 ±0.0049	0.2835 ±0.0050	0.1960 ±0.0104

Notes: Concentrations of all feed solutions: maltodextrin: 10% (w/w); gum Arabic: 10% (w/w); RSM (reconstituted skim milk): 10% (w/w); MWM (mixed wall material): 10% (w/w).

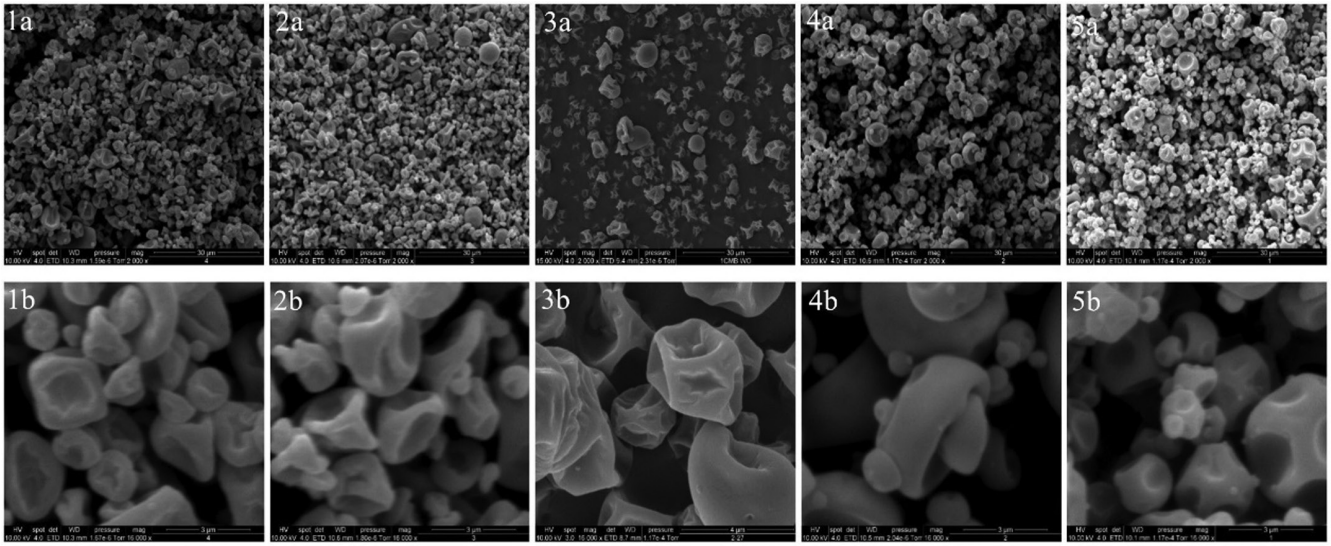

FIGURE 2 Scanned electron micrographs of spray-dried DPC16 microcapsules. (1a) 10% RSM (w/w) microcapsules spray-dried at 160°C/80°C inlet/outlet temperatures and magnified at ×2000; (1b) 10% RSM (w/w) microcapsules spray-dried at 160°C/80°C and magnified at ×16,000; (2a,b) 10% RSM (w/w) microcapsules spray-dried at 180°C/100°C (inlet/outlet temperatures) and magnified at ×2000 and ×16,000, respectively; (3a,b) 10% MWM (w/w) microcapsules spray-dried at 160°C/80°C (inlet/outlet temperatures) and magnified at ×2000 and ×16,000, respectively; (4a,b) 10% gum Arabic (w/w) microcapsules spray-dried at 160°C/80°C (inlet/outlet temperatures) and magnified at ×2000 and ×16,000, respectively; (5a,b) 10% maltodextrin (w/w) microcapsules spray-dried at 160°C/80°C (inlet/outlet temperatures) and magnified at ×2000 and ×16,000, respectively. The scanned electron micrographs were captured by the FEI electron optics, quanta 200 (The Netherlands)

variable sizes, and had wrinkled surfaces. The particle surface of powders made from RSM and spray-dried at the inlet/outlet temperatures of 180°C/100°C (Figure 2-2a,b) was similar to the sample produced at 160°C/80°C. Most microcapsules made from the MWM and spray-dried at 160°C/80°C (Figure 2-3a,b) were characterized by dents or concavities on the surfaces, which were similar to the surface of samples made from the RSM (Figure 2-1a,b). Some of the MWM microcapsules had smooth surfaces which were similar to those made using gum Arabic (Figure 2-4a,b). However, fewer large particles were present among the MWM microcapsules compared with RSM microcapsules.

Compared to the doughnut-like particles made from the MWM (Figure 2-3a,b), the DPC16 microcapsules made from gum Arabic alone (Figure 2-4a,b) as wall material had more dents on the surface, but still smoother than RSM microcapsules which were wrinkled (Figure 2-1a,b). For maltodextrin microcapsules (Figure 2-5a,b), numerous dents were observed on the surface of most particles and even small particles had wrinkled surfaces.

4 | DISCUSSION

Spray-drying process can cause conformation, dehydration, and transition of cells from glassy state to rubbery state (García, 2011). Cell death during spray drying was reported to be mainly caused by changes in the configuration and profile of cellular lipids in the cell wall and cytoplasmic membrane. The unsaturated fatty acids and lipolysis in cells can be damaged by oxidation (Teixeira et al., 1996). The encapsulation wall material which aims to protect cellular membrane lipids has been reported to be a determinant factor for the survival of probiotic cells due to their differences in thermal conductivity and diffusivity (Lian et al., 2002).

The high EE of all wall materials (>80.00%) for encapsulation of DPC16 observed in this study agreed with previous research (Manojlović et al., 2010; Sohail et al., 2013; Ying et al., 2012). Maltodextrin, RSM, and gum Arabic have long been used as wall materials to encapsulate probiotics and are effective (Manojlović et al., 2010; Sohail et al., 2013). In this study, the MWM also had a high EE of 93.97±3.44% which was in agreement with Ying

et al. (2012) who reported that a combination of protein, prebiotic and small sugar can provide good protection to cells during spray drying. In addition, mixing gum Arabic and other wall materials rather than using the gum alone may improve the EE of probiotic cells (Fazilah et al., 2019).

In this study, the EE of maltodextrin as a wall material was lower than skim milk, which also agreed with the results of Liao et al. (2017). According to Pinto et al. (2015), maltodextrin with low dextrose equivalent (DE) has high glass-transition temperature which is not capable of replacing water molecules on cell membrane due to its high molecular weight. Thus, maltodextrin with low DE is unable to maintain microbial cell structure when the water molecules on the cell membrane are removed during spray drying. The DE of maltodextrin used in this study was relatively low at 10. During the spray-drying process, it is vital to maintain the structure and function of bacterial cell membrane when cells are exposed to high temperatures. Maltodextrin is therefore less effective to protect the cells during spray drying (Pinto et al., 2015). In addition, maltodextrin has a low emulsifying ability which can result in the leakage of cells (Cano-Higuita et al., 2015). Maltodextrin also tends to penetrate the cell membrane and is less likely to bind to the cell membrane (Semyonov et al., 2010). The low emulsifying ability of maltodextrin in the "cell-in-water" system, and poor cell membrane-binding capacity of maltodextrin can be explained by the nonspecific adhesion of bacteria, which is dominated by the molecular hydrophobicity of the wall material, Van der Waals forces, and electrostatic attraction. DPC16 is a gram-positive bacteria and there are many teichoic acids on the cell membrane (Neuhaus & Baddiley, 2003). Meanwhile, both RSM and gum Arabic contain protein components. The amino group in the protein and teichoic acid undergo acid–base neutralization reaction. Then the ammonium formed is positively charged, and the teichoic acid is negatively charged (due to the loss of a proton), forming an ion pair. This ionic bond is stable. Whereas, the hydroxyl group in maltodextrin is weakly acidic, while teichoic acid is stronger acidic so the interaction between teichoic acid and maltodextrin is quite unstable, which leads to the poor cell-binding capacity and emulsion ability of maltodextrin in the "cell-in-water" system. The slow deposition of maltodextrin in the chamber of the spray-dryer also contributes to the low EE of maltodextrin because cells would be exposed at the inlet higher temperature of the dryer for a longer period (Langrish et al., 2007).

Gum Arabic was the only wall material that had a significant difference in EE from RSM in this study ($p < .05$). The relatively low EE of gum Arabic might be attributed to its high viscosity and poor thermo-protective effect on cells at lower outlet temperature (<100°C) (Desmond et al., 2002).

RSM has been reported to have outstanding protective properties for probiotic bacteria and therefore, it is the most frequently used wall protective material for microencapsulation of bacteria by spray drying (Fritzen-Freire et al. 2013; Gul, 2017). The higher survival of DPC16 cells encapsulated in RSM compared to those of gum Arabic and maltodextrin during spray drying at 160°C/80°C might be due to the presence of lactose which can replace cellular water molecules during spray drying, thereby maintaining cell structure (Ying et al., 2012). In addition, compared to gum Arabic which was also added to the MWM, RSM had lower viscosity at the same temperature and concentration (Phillips & Williams, 2009; Schmidt & Smith, 1992). Thus, encapsulation wall material with low viscosity is preferred because it promotes the atomization of feed solution, thereby protecting cells from thermal effect and dehydration due to early formation of crust (Anandharamakrishnan, 2015).

With 10% RSM (w/w) as wall material, the EE decreased markedly at an elevated inlet/outlet temperatures of 180°C/100°C (83.85 ± 1.50%) compared to the results obtained using 160°C/80°C (98.06 ± 1.73%). The drying temperatures were thus important factors for the survival of cells, which was in agreement with previous research (Anandharamakrishnan, 2015; Würth et al., 2018).

The level of water activity of the produced powders were arranged in the following order: gum Arabic < maltodextrin < skim milk powder at 160°C/80°C which agreed with the results of Kalušević, Lević, Čalija, Milić, et al. (2017). The study by the previous researchers used wall materials (gum Arabic, maltodextrin and skim milk powder) to encapsulate grape skin extract at inlet/outlet temperatures of 140°C/65°C. Water activity of the spray-dried microcapsules made from different wall materials ranged from 0.24 to 0.28 following the order of gum Arabic < maltodextrin < skim milk. However, under similar spray-drying inlet/outlet temperatures, a_w of the spray-dried microcapsule powders made from the three wall materials ranged from 0.31 to 0.33 and no differences ($p < .05$) were reported when the wall materials were used to encapsulate soybean coat extract (Kalušević, Lević, Čalija, Pantić, et al., 2017).

When powders were spray-dried at the inlet/outlet temperatures of 160°C/80°C, RSM had the highest EE (98.06 ± 0.86%) and DPC16 microcapsule made from this wall material had the highest a_w (0.284 ± 0.005) (Table 2). For powders containing probiotics, water activity <0.25 is recommended to avoid the mortality of bacteria caused by stimulating metabolism due to higher mobility of water molecules (Albadran et al., 2015). However, RSM microcapsules produced in this study contained higher a_w than this range. The higher a_w of RSM microcapsules than microcapsules made from gum Arabic and MWM (which also contained gum Arabic) produced at 160°C/80°C might be associated with the viscosity of the feed solutions. Gum Arabic can increase the viscosity of the feed solution which leads to difficulties in atomization (Anandharamakrishnan, 2015; Schmidt & Smith, 1992). Thus, our spray-dryer (Saurin SL-10) produced the gum Arabic microcapsules and MWM microcapsules at a low flow rate with inlet/outlet temperatures (160°C/80°C). The decrease in mass flow rate reduces water content in the powders produced due to the presence of gum Arabic and MWM droplets for a longer time at the inlet of the dryer (160°C) leading to more evaporation of water. The a_w of RSM microcapsules was also higher than the maltodextrin microcapsules, although maltodextrin does not have a relatively higher viscosity (Dokic et al., 1998). This might be caused by the slower deposition of maltodextrin than RSM in the spray-drying chamber which leads to a longer drying time of a particle. Langrish et al. (2007) reported that the deposition process is likely to be initiated by the

adhesion of particles on the clean surface of the spray-drying chamber. After the chamber is covered by particles, cohesion of particles onto other particles is likely to happen, which controls the process of deposition. However, for maltodextrin, the cohesion of particles occurs more rapidly than adhesion of particles to the chamber, thus the deposition (adhesion) of maltodextrin is slow because of a lack of particles on the wall of the chamber.

The a_w of the DPC16 microcapsule powder made from the MWM (10%, w/w) was 0.196 ± 0.010, lower than that of the powder (0.284 ± 0.005) made from skim milk (10%, w/w). Except for the high viscosity caused by the presence of gum Arabic, the low a_w was probably attributed to the presence of inulin and sucrose (Avila-Reyes et al., 2014). The shorter chains of inulin and higher number of hydroxyls can bind water molecules that are not removed during the spray-drying process which lowers the a_w of powder made from the MWM (Avila-Reyes et al., 2014). Sucrose and sodium chloride are the most commonly used osmotic agents which decrease the a_w of food (Kim & Toledo, 1987).

The a_w of powder made from RSM and spray-dried at 180°C/100°C was 0.200 ± 0.004, lower than the sample spray-dried at 160°C/80°C (0.284 ± 0.005). This result suggested that the a_w of the powder was related to the drying temperatures. An increase in the inlet/outlet temperatures decreased the a_w ($p < .05$) which agreed with previous studies (Baysan et al., 2019; Teanpaisan et al., 2012). Water activity is vitally important for the quality of spray-dried powder during storage because it affects the rate of physical deterioration such as caking and stickiness (Hedegaard & Skibsted, 2013). The reactions such as non-enzymatic browning and the oxidation of lipid and protein in cell membrane which lead to cell death are also affected by the a_w (Hedegaard & Skibsted, 2013). Thus, low a_w of DPC16 microcapsules is desirable for the storage of powders.

The surface appearance of DPC16 microcapsules made from RSM and spray-dried at 160°C/80°C agreed with the study by Desmond et al. (2002). The particles had a wrinkled surface as they were covered by protein (Xu et al., 2012). According to previous studies, the dense surfaces containing high protein content are formed during drying because of the migration of surface-active protein (Fyfe et al., 2011; Shrestha et al., 2007).

The appearance of RSM microcapsules spray-dried at different inlet/outlet temperatures was in contrast to the study by Maa et al. (1997), who reported increased dents/concavities on particle surfaces and decreased spherical particles as the outlet temperature increased. The similarity (shape and dents/concavities) of microcapsules made from MWM and RSM was probably attributed to the presence of whey protein isolate in the MWM (Maciel et al., 2014). The morphology of probiotic microcapsules made from skim milk was similar to those made from sweet whey (Maciel et al., 2014). In the formulation of MWM, there was also gum Arabic, inulin, and sucrose. The addition of inulin into the feed solution did not appear to affect the morphology of spray-dried powders (Fritzen-Freire et al., 2012). However, results showed that the morphology of spray-dried powders was affected by the addition of gum Arabic (Fazilah et al., 2019). The doughnut-like particles with smooth surface and few dents or roughness among MWM microcapsules were presumed to be particles consisting of gum Arabic as reported by Fazilah et al. (2019). Previous studies have reported that a combination of wall materials containing gum Arabic contributed to smoother particle surface compared to using gum Arabic alone (Fazilah et al., Lian et al., 2002), which was also observed in the current study.

The powder made from the MWM had fewer larger particles (Figure 2-3a,b) than the powder made from RSM (Figure 2-1a,b), probably due to the presence of gum Arabic in MWM, which increased the viscosity of the feed solution and prolonged the drying time of the droplets, thereby decreasing the size of the spray-dried microcapsules.

Gum Arabic microcapsules had dents on their surface but were still smoother than RSM microcapsules. Similar results were also reported by other studies (Bhusari et al., 2014; Ferrari et al., 2012; Rascón et al., 2011). The dents were most likely caused by high water evaporation rate during spray drying resulting in the shrinkage of particles (Kuck & Noreña, 2016).

The surface of DPC16 microcapsules made from maltodextrin had numerous dents and was similar to results reported by Rodríguez-Huezo et al. (2007). Powder particles with dents were reported to be difficult to dissolve (Reyes et al., 2018), thus microcapsules with smooth surfaces are desirable.

5 | CONCLUSIONS

The encapsulation of *L. reuteri* DPC16 using RSM by spray drying at elevated inlet/outlet temperatures (180°C/100°C) resulted in lower EE and water activity compared to RSM microcapsules spray-dried at 160°C/80°C ($p < .05$). At lower inlet/outlet temperatures (160°C/80°C), RSM had the highest EE, and highest a_w of the spray-dried microcapsules which was undesirable. Gum Arabic microcapsules had the lowest EE and a_w ($p < .05$). However, the MWM comprising gum Arabic, whey protein isolate, inulin, and sucrose produced microcapsules with relatively high EE, low a_w, and some microcapsules with smoother surfaces which could improve powder solubility. Thus, RSM and the MWM may be promising encapsulation wall materials for the probiotic *L. reuteri* DPC16 by spray drying.

AUTHOR CONTRIBUTIONS

Fang Wang: Investigation; methodology. **Anthony N.Mutukumira:** Conceptualization; investigation; methodology; resources; supervision; validation.

ACKNOWLEDGMENTS

The authors thank the Massey University for funding and Bioactives Research Laboratory Limited New Zealand for the supply of L. reuteri DPC16. Open access publishing facilitated by Massey University, as part of the Wiley - Massey University agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Anthony N. Mutukumira https://orcid.org/0000-0003-3189-2120

REFERENCES

Albadran, H. A., Chatzifragkou, A., Khutoryanskiy, V. V., & Charalampopoulos, D. (2015). Stability of probiotic *Lactobacillus plantarum* in dry microcapsules under accelerated storage conditions. *Food Research International*, *74*, 208–216.

Anandharamakrishnan, C. (2015). *Spray drying techniques for food ingredient encapsulation*. John Wiley and Sons.

Avila-Reyes, S. V., Garcia-Suarez, F. J., Jiménez, M. T., San Martín-Gonzalez, M. F., & Bello-Perez, L. A. (2014). Protection of *Lactobacillus rhamnosus* by spray-drying using two prebiotics colloids to enhance the viability. *Carbohydrate Polymers*, *102*, 423–430. https://doi.org/10.1016/j.carbpol.2013.11.033

Baysan, U., Elmas, F., & Koç, M. (2019). The effect of spray drying conditions on physicochemical properties of encapsulated propolis powder. *Journal of Food Process Engineering*, *42*, e13024. https://doi.org/10.1111/jfpe.13024

Bhusari, S., Muzaffar, K., & Kumar, P. (2014). Effect of carrier agents on physical and microstructural properties of spray dried tamarind pulp powder. *Powder Technology*, *266*, 354–364.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*, *104*, 467–483.

Călinoiu, L. F., Ştefănescu, B. E., Pop, I. D., Muntean, L., & Vodnar, D. C. (2019). Chitosan coating applications in probiotic microencapsulation. *Coatings*, *9*, 194.

Cano-Higuita, D., Malacrida, C., & Telis, V. (2015). Stability of curcumin microencapsulated by spray and freeze drying in binary and ternary matrices of maltodextrin, gum Arabic and modified starch. *Journal of Food Processing and Preservation*, *39*, 2049–2060.

Champagne, C. P., & Fustier, P. (2007). Microencapsulation for the improved delivery of bioactive compounds into foods. *Current Opinion in Biotechnology*, *18*, 184–190.

Desmond, C., Ross, R., O'callaghan, E., Fitzgerald, G., & Stanton, C. (2002). Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. *Journal of Applied Microbiology*, *93*, 1003–1011.

Dokic, P., Jakovljevic, J., & Dokic-Baucal, L. (1998). Molecular characteristics of maltodextrins and rheological behaviour of diluted and concentrated solutions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *141*, 435–440.

FAO/WHO. (2002). Joint FAO/WHO working group report on drafting guidelines for the evaluation of probiotics in food ftp. fao. org/es/esn/food/wgreport2.Pdf. Food and Agricultural Organisation/World Health Organisation of The United Nations, Rome.Italy.

Fazilah, N. F., Hamidon, N. H., Ariff, A. B., Khayat, M. E., Wasoh, H., & Halim, M. (2019). Microencapsulation of *Lactococcus lactis* Gh1 with gum Arabic and *Synsepalum dulcificum* via spray drying for potential inclusion in functional yogurt. *Molecules*, *24*, 1422.

Ferrari, C. C., Germer, S. P. M., Alvim, I. D., Vissotto, F. Z., & de Aguirre, J. M. (2012). Influence of carrier agents on the physicochemical properties of blackberry powder produced by spray drying. *International Journal of Food Science & Technology*, *47*, 1237–1245.

Fritzen-Freire, C. B., Prudêncio, E. S., Amboni, R. D., Pinto, S. S., Negrão-Murakami, A. N., & Murakami, F. S. (2012). Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. *Food Research International*, *45*, 306–312.

Fritzen-Freire, C. B., Prudêncio, E. S., Pinto, S. S., Muñoz, I. B., & Amboni, R. D. M. C. (2013). Effect of microencapsulation on survival of Bifidobacterium BB-12 exposed to simulated gastrointestinal conditions and heat treatments. *LWT - Food Science and Technology*, *50*(1), 39–44 https://doi.org/10.1016/j.lwt.2012.07.037

Fyfe, K., Kravchuk, O., Nguyen, A. V., Deeth, H., & Bhandari, B. (2011). Influence of dryer type on surface characteristics of milk powders. *Drying Technology*, *29*, 758–769.

Gaiani, C., Burgain, J., & Scher, J. (2013). Surface composition of food powders. In B. Bhandari, N. Bansal, M. Zhang, & P. Schuck (Eds.), *Handbook of food powders* (pp. 339–378). Woodhead Publishing. https://doi.org/10.1533/9780857098672.2.339

García, A. H. (2011). Anhydrobiosis in bacteria: From physiology to applications. *Journal of Biosciences*, *36*, 939–950.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International*, *40*, 1107–1121.

Gul, O. (2017). Microencapsulation of *Lactobacillus casei* Shirota by spray drying using different combinations of wall materials and application for probiotic dairy dessert. *Journal of Food Processing and Preservation*, *41*, e13198.

Hedegaard, R., & Skibsted, L. H. (2013). Shelf-life of food powders. In B. Bhandari, N. Bansal, M. Zhang, & P. Schuck (Eds.), *Handbook of food powders* (pp. 409–434). Woodhead Publishing. https://doi.org/10.1533/9780857098672.2.409

Ishibashi, N. (1993). Bifidobacteria: Research and development in Japan. *Food Technology*, *47*, 126–135.

Ivanovska, T. P., Zhivikj, Z., Mladenovska, K., & Petrushevska-Tozi, L. (2015). Influence of oligofructose-enriched inulin on survival of microencapsulated *Lactobacillus casei* 01 and adhesive properties of synbiotic microparticles. *Makedonsko Farmacevtski Bilten*, *61*, 35–43.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2008). *Modern food microbiology*. Springer Science and Business Media.

Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: Technology and potential applications. *Current Issues in Intestinal Microbiology*, *3*, 39–48.

Kalušević, A., Lević, S., Čalija, B., Pantić, M., Belović, M., Pavlović, V., Bugarski, B., Milić, J., Žilić, S., & Nedović, V. (2017). Microencapsulation of anthocyanin-rich black soybean coat extract by spray drying using maltodextrin, gum Arabic and skimmed milk powder. *Journal of Microencapsulation*, *34*, 475–487.

Kalušević, A., Lević, S. M., Čalija, B. R., Milić, J. R., Pavlović, V. B., Bugarski, B. M., & Nedović, V. A. (2017). Effects of different carrier materials on physicochemical properties of microencapsulated grape skin extract. *Journal of Food Science and Technology*, *54*, 3411–3420.

Kim, M., & Toledo, R. (1987). Effect of osmotic dehydration and high temperature fluidized bed drying on properties of dehydrated rabbiteye blueberries. *Journal of Food Science*, *52*, 980–984.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yoghurt. *International Dairy Journal*, *13*, 3–13.

Kuck, L. S., & Noreña, C. P. Z. (2016). Microencapsulation of grape (*Vitis labrusca* var. Bordo) skin phenolic extract using gum Arabic, polydextrose, and partially hydrolyzed guar gum as encapsulating agents. *Food Chemistry*, *194*, 569–576.

Lakkis, J. M. (2007). *Encapsulation and controlled release technologies in food systems*. Wiley Online Library.

Langrish, T. A. G., Chan, W. C., & Kota, K. (2007). Comparison of maltodextrin and skim milk wall deposition rates in a pilot-scale spray dryer. *Powder Technology*, *179*, 84–89.

Lian, W. C., Hsiao, H. C., & Chou, C. C. (2002). Survival of bifidobacteria after spray-drying. *International Journal of Food Microbiology*, *74*, 79–86.

Liao, L., Wei, X., Gong, X., Li, J., Huang, T., & Xiong, T. (2017). Microencapsulation of *Lactobacillus casei* LK-1 by spray drying related to its stability and in vitro digestion. *LWT - Food Science and Technology*, *82*, 82–89.

Maa, Y. F., Costantino, H. R., Nguyen, P. A., & Hsu, C. C. (1997). The effect of operating and formulation variables on the morphology of spray-dried protein particles. *Pharmaceutical Development and Technology*, *2*, 213–223.

Maciel, G., Chaves, K., Grosso, C., & Gigante, M. (2014). Microencapsulation of *Lactobacillus acidophilus* La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. *Journal of Dairy Science*, *97*, 1991–1998.

Manojlović, V., Nedović, V. A., Kailasapathy, K., & Zuidam, N. J. (2010). Encapsulation of probiotics for use in food products. In N. J. Zuidam & V. Nedovic (Eds.), *Encapsulation technologies for active food ingredients and food processing* (pp. 269–302). Springer.

Mutukumira, A. N., Ang, J., & Lee, S. J. (2015). Microencapsulation of probiotic bacteria. In M. T. Liong (Ed.), *Beneficial microorganisms in food and nutraceuticals* (pp. 63–80). Springer. https://doi.org/10.1007/978-3-319-23177-8

Neuhaus, F. C., & Baddiley, J. (2003). A continuum of anionic charge: Structures and functions of D-alanyl-teichoic acids in gram-positive bacteria. *Microbiology and Molecular Biology Reviews*, *67*, 686–723.

Pimentel, T. C., da Costa, W. K. A., Barão, C. E., Rosset, M., & Magnani, M. (2021). Vegan probiotic products: A modern tendency or the newest challenge in functional foods. *Food Research International*, *140*, 110033. https://doi.org/10.1016/j.foodres.2020.110033

Pinto, S. S., Fritzen-Freire, C. B., Benedetti, S., Murakami, F. S., Petrus, J. C. C., Prudêncio, E. S., & Amboni, R. D. (2015). Potential use of whey concentrate and prebiotics as carrier agents to protect *Bifidobacterium* BB-12 microencapsulated by spray drying. *Food Research International*, *67*, 400–408.

Phillips, G. O., & Williams, P. A. (2009). *Handbook of hydrocolloids*. CRC press.

Prüsse, U., Bilancetti, L., Bučko, M., Bugarski, B., Bukowski, J., Gemeiner, P., Lewińska, D., Manojlovic, V., Massart, B., & Nastruzzi, C. (2008). Comparison of different technologies for alginate beads production. *Chemical Papers*, *62*, 364–374.

Rajam, R., & Anandharamakrishnan, C. (2015). Microencapsulation of *Lactobacillus plantarum* (MTCC 5422) with fructooligosaccharide as wall material by spray drying. *Food Science and Technology*, *60*, 773–780. https://doi.org/10.1016/j.lwt.2014.09.062

Rascón, M. P., Beristain, C. I., García, H. S., & Salgado, M. A. (2011). Carotenoid retention and storage stability of spray-dried encapsulated paprika oleoresin using gum Arabic and soy protein isolate as wall materials. *LWT - Food Science and Technology*, *44*(2), 549–557.

Reyes, V., Chotiko, A., Chouljenko, A., & Sathivel, S. (2018). Viability of *Lactobacillus acidophilus* NRRL B-4495 encapsulated with high maize starch, maltodextrin, and gum arabic. *LWT*, *96*, 642–647.

Rodríguez-Huezo, M., Durán-Lugo, R., Prado-Barragán, L., Cruz-Sosa, F., Lobato-Calleros, C., Alvarez-Ramírez, J., & Vernon-Carter, E. (2007). Pre-selection of protective colloids for enhanced viability of *Bifidobacterium bifidum* following spray-drying and storage, and evaluation of aguamiel as thermoprotective prebiotic. *Food Research International*, *40*, 1299–1306.

Sahin, T., Aydin, S., Yüksel, O., Bostanci, H., Akyürek, N., Memis, L., & Basaran, N. (2007). Effects of the probiotic agent *Saccharomyces boulardii* on the DNA damage in acute necrotizing pancreatitis induced rats. *Human and Experimental Toxicology*, *26*, 653–661.

Schmidt, K. A., & Smith, D. E. (1992). Rheological properties of gum and milk protein interactions. *Journal of Dairy Science*, *75*, 36–42.

Semyonov, D., Ramon, O., Kaplun, Z., Levin-Brener, L., Gurevich, N., & Shimoni, E. (2010). Microencapsulation of *Lactobacillus paracasei* by spray freeze drying. *Food Research International*, *43*, 193–202.

Shrestha, A. K., Howes, T., Adhikari, B. P., Wood, B. J., & Bhandari, B. R. (2007). Effect of protein concentration on the surface composition, water sorption and glass transition temperature of spray-dried skim milk powders. *Food Chemistry*, *104*, 1436–1444.

Sohail, A., Turner, M. S., Coombes, A., & Bhandari, B. (2013). The viability of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* NCFM following double encapsulation in alginate and maltodextrin. *Food and Bioprocess Technology*, *6*, 2763–2769.

Tan, J., & Balasubramanian, B. M. (2017). Particle size measurements and scanning electron microscopy (SEM) of cocoa particles refined/conched by conical and cylindrical roller stone melangers. *Journal of Food Engineering*, *212*, 146–153. https://doi.org/10.1016/j.jfoodeng.2017.05.033

Teanpaisan, R., Chooruk, A., Wannun, A., Wichienchot, S., & Piwat, S. (2012). Survival rates of human-derived probiotic *Lactobacillus paracasei* SD1 in milk powder using spray drying. *Sonklanakarin Journal of Science and Technology*, *34*, 247.

Teixeira, P., Castro, H., & Kirby, R. (1996). Evidence of membrane lipid oxidation of spray-dried *Lactobacillus bulgaricus* during storage. *Letters in Applied Microbiology*, *22*, 34–38.

Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., & Kopsahelis, N. (2019). Probiotics in food systems: Significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients*, *11*, 1591.

Thantsha, M. S., Cloete, T. E., Moolman, F. S., & Labuschagne, P. W. (2009). Supercritical carbon dioxide interpolymer complexes improve survival of *B. longum* bb-46 in simulated gastrointestinal fluids. *International Journal of Food Microbiology*, *129*, 88–92.

Würth, R., Foerst, P., & Kulozik, U. (2018). Effects of skim milk concentrate dry matter and spray drying air temperature on formation of capsules with varying particle size and the survival microbial cultures in a microcapsule matrix. *Drying Technology*, *36*, 93–99.

Xu, Y. Y., Howes, T., Adhikari, B., & Bhandari, B. (2012). Investigation of relationship between surface tension of feed solution containing various proteins and surface composition and morphology of powder particles. *Drying Technology*, *30*, 1548–1562.

Ying, D., Sun, J., Sanguansri, L., Weerakkody, R., & Augustin, M. A. (2012). Enhanced survival of spray-dried microencapsulated *Lactobacillus rhamnosus* GG in the presence of glucose. *Journal of Food Engineering*, *109*, 597–602. https://doi.org/10.1016/j.jfoodeng.2011.10.017

How to cite this article: Wang, F., & Mutukumira, A. N. (2022). Microencapsulation of *Limosilactobacillus reuteri* DPC16 by spray drying using different encapsulation wall materials. *Journal of Food Processing and Preservation*, *46*, e16880. https://doi.org/10.1111/jfpp.16880