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Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: Technological characterization, storage stability and survival after *in vitro* digestion

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ABSTRACT

We evaluated sodium alginate, chitosan and hydroxypropyl methylcellulose (HPMC) as co-encapsulants for spray dried *Lactobacillus acidophilus* NCIMB 701748 by assessing their impact on cell viability and physicochemical properties of the dried powders, viability over 35 days of storage at 25 °C and survival after simulated digestion. Fibres were added to a control carrier medium containing whey protein concentrate, D-glucose and maltodextrin. Sodium alginate and HPMC did not affect cell viability but chitosan reduced viable counts in spray dried powders, as compared to the control. Although chitosan caused large losses of viability during spray-drying, these losses were counteracted by the excellent storage stability compared to control, sodium alginate and HPMC, and the overall effect became positive after the 35-day storage. Chitosan also improved survival rates in simulated GI conditions, however no single fibre could improve *L. acidophilus* NCIMB 701748 viability in all steps from production through storage and digestion.

1. Introduction

According to FAO/WHO (2001) probiotics are defined as live organisms that when administered in adequate amounts (>10^7 cfu/g of finished product) confer health benefits to the host. The functional and physiological action of probiotics is part of a very active field of research and so far the main health benefits proposed to be associated with probiotics are the improvement of gastrointestinal function and the immune system, control of lactose intolerance symptoms, reduction of blood cholesterol levels and prevention of carcinogenesis (Macfarlane & Cummings, 1999). To date many systems have been developed in order to deliver probiotics, including fermented and non-fermented dairy products, fruit juices, emulsions, breakfast cereals, cereal bars, ice creams, cheeses and their derivatives (Antunes, Cazetto, & Bolini, 2005; Cruz, Antunes, Sousa, Faria, & Saad, 2009; dos Santos Leandro, de Araújo, da Conceição, de Moraes, & de Carvalho,

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2013; Mantzouridou, Spanou, & Kiosseoglou, 2012; Saarela, Virkajarvi, Alakomi, Sigvart-Mattila, & Matto, 2006; Ying et al., 2013). However, except for dairy products, the addition of probiotics to most food products requires pre-encapsulation of bacterial cells to maintain viability under the harsh chemical and physical conditions presented in various food matrices (Burgain, Gaiani, Linder, & Scher, 2011; Nazzaro, Fratianni, Coppola, Sada, & Orlando, 2009; Ying et al., 2013).

The viability of bacteria in probiotic foods can be affected by harsh heat treatment and mechanical processing, storage at room temperature, the physical state of the food matrix and the chemical micro-environment of the bacteria (Fu & Chen, 2011). Entrapment of probiotic cells in complex hydrocolloid matrices by mechanical or physicochemical processes such as emulsification, extrusion, gelation and coacervation has previously been used to reduce the loss of viability during processing and storage. Furthermore a range of processes have been used to obtain dry formulations with prolonged shelf-life, the most common being freeze drying, vacuum drying and spray drying. Spray drying is recognised as one of the most convenient in terms of energy requirements, cost and process yield, whilst thermal and osmotic damage to the probiotic cells has to be minimised by carefully optimizing operating conditions and carrier media composition (Behboudi-Jobbehdar, Soukoulis, Yonekura, & Fisk, 2013; Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2013).

Probiotic species and strain, along with thermal and osmotic adaptation, and the culture growth stage (logarithmic or stationary) have all been reported to impact on the viability of probiotics during spray drying (Champagne, Ross, Saarela, Hansen, & Charalampopoulos, 2011; Corcoran, Ross, Fitzgerald, & Stanton, 2004; Fu & Chen, 2011). Whilst it is not always possible to simply opt for a process stable strain, carrier media such as those based on maltodextrin and proteins can be further combined with thermoprotective compounds to minimise heat related injuries during spray drying (Lapsiri, Bhandari, & Wanchaitanawong, 2012). While the exact mechanisms describing the functionality of these ingredients are not completely understood, it is generally accepted that their ability to minimise structural changes of cell membranes during phase transitions (crystalline to rubbery) plays a major role in improving cellular integrity (Ananta, Volkert, & Knorr, 2005; Fu & Chen, 2011; Gardiner et al., 2000). Additional thermoprotection can be achieved, in some situations, through the addition of free radical scavengers (e.g. whey proteins) or by reducing water mobility through the cell membranes and the cell wall thereby modulating dehydration upon heating (Ying, Sun, Sanguansri, Weerakkody, & Augustin, 2012).

With respect to spray dried microcapsules containing soluble dietary fibre, studies have reported the inclusion of arabic gum, acacia gum, sodium alginate and pectin into the bulk drying medium (Bustos & Bórquez, 2013; Desmond, Ross, O'Callaghan, Fitzgerald, & Stanton, 2002; Rajam, Karthik, Parthasarathi, Joseph, & Anandharamakrishnan, 2012). Desmond et al. (2002) reported a 10-fold improvement of survival after spray drying of *Lactobacillus paracasei* by replacing half of the skim milk powder by acacia gum in the growth/drying medium. In another study, the use of binary soluble fibre–maltodextin blends (acacia gum or fibersol) did not furnish any thermoprotection to *Lactobacillus plantarum* throughout spray drying compared to trehalose or soy protein concentrate (Lapsiri et al., 2012).

Once successfully incorporated into a carrier matrix, probiotics should ideally retain their viability during storage and ultimately survive the low pH, digestive enzymes and bile salts of the human gastrointestinal tract and reach the colon in order to confer their beneficial effects to the host. Although, again, the resistance to gastrointestinal conditions is species- and strain-dependent (Monteagudo-Mera et al., 2012), the choice of carrier matrix can improve survival and significantly increase the number of viable bacteria reaching the colon. The incorporation of acid-labile bacteria into alginate (Corbo, Bevilacqua, Gallo, & Speranza, 2013) or alginate–chitosan microbeads (Li, Chen, Sun, Park, & Cha, 2011) conferred effective protection against the low-pH gastric juice, and the presence of chitosan further improved the recovery of viable cells at the end of the simulated digestion (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2011). While micro-beads are valuable vehicles for the controlled release of drugs and probiotics, their granular nature (1–2 mm diameter) would be an obstacle for incorporation in most food systems.

Spray drying produces powders with particle sizes in the micrometre scale, which would have a smoother mouth feel than micro-beads and should allow the addition of probiotics to a wider range of foods. Currently the evidence supporting the use of soluble fibres as co-components for stabilising probiotic spray dried powders is still relatively scarce, and often the previously published studies do not offer a clear insight into how the physicochemical properties of the carrier matrix are correlated with the loss/enhancement of viability during spray drying, storage and gastrointestinal conditions. We therefore evaluated three soluble dietary fibres (sodium alginate, chitosan and hydroxypropyl methylcellulose) as potential co-encapsulants for spray dried powders containing *L. acidophilus* NCIMB 701748, and investigated the impact of soluble fibre on the physicochemical properties of the dried powder and the viability of probiotic cells after spray drying, room temperature storage and *in vitro* digestion.

2. Methods and materials

2.1. Materials

Lactobacillus acidophilus NCIMB 701748 was purchased as freeze-dried culture from NCIMB Ltd. (Aberdeen, Scotland, UK). Hydroxypropyl methyl cellulose (HPMC), chitosan (Poly-D-glucosamine, deacetylated chitin), lactic acid, were obtained from Sigma–Aldrich (Loughborough, UK). Sodium alginate (S20933), maltodextrin 15 DE (C* Dry MD 01910) and whey protein concentrate (Lacprodan® DI-8090) were kindly provided by FMC Biopolymer (Drammen, Norway), Cargill Ltd. (Manchester, UK) and Arla A/S (Viby, Denmark), respectively.

Pepsin from porcine gastric mucosa (Sigma P7125, 400–800 U/mg), ox-bile (Fluka 70168) and pancreatin from porcine pancreas (Sigma P1750), were purchased from Sigma–Aldrich (Loughborough, UK). All other reagents were of analytical grade, unless specified otherwise.

2.2. Culture frozen storage and growth conditions

The freeze-dried stock of *Lactobacillus acidophilus* NCIMB 701748 was suspended in a small amount of medium, streaked onto MRS-agar plate and incubated at 37 °C with 5% CO_2 for 24 h. Colonies were collected with a sterilized loop, suspended in the cryoprotectant medium of Roti®-Store systems (Roti®-Store, Carl-Roth GmbH, Karlsruhe, Germany) and the glass bead cultures were stored in a freezer at −80 °C until use (Ananta et al., 2005).

Beads of deep frozen cultures were transferred (one bead per 100 mL) to MRS broth (Oxoid Ltd., Basingstoke, UK) and incubated for 48 h at 37 °C, under anaerobic conditions in plastic jars containing AnaeroGen (Oxoid Ltd., Basingstoke, UK). Under aseptic conditions, the MRS broth cell suspension was divided into 50 mL polypropylene centrifuge tubes (Sarstedt GmbH, Leicester, UK) and centrifuged at 3000*g* for 5 min. Supernatants were discarded and cell pellets were washed with 50 mL phosphate buffered saline (Dulbecco A PBS, Oxoid Ltd., Basingstoke, UK), and re-centrifuged. After discarding the supernatants, the collected bacterial cell pellets were suspended in the drying carrier media.

2.3. Preparation of drying carrier media

In all cases the concentration of soluble fibre was chosen as the maximum level that could practically be atomized in the spray dryer, the composition of drying carrier media is given in Table 1. Sodium alginate, HPMC and chitosan/lactic acid were dissolved in water at room temperature by magnetic stirring. Whey protein concentrate, maltodextrin and D-glucose were dispersed in water and allowed to hydrate for 1 h using a magnetic stirrer. The protein/carbohydrate and soluble fibre solutions were heat-treated separately (to avoid phase separation due to fibre–protein interaction) at 75 °C for 10 min and cooled to room temperature. Six *L. acidophilus* NCIMB 701748 pellets were suspended into 100 mL of the protein/carbohydrate suspension and combined with an equal volume of soluble fibre solution prior to use. Final count of *L. acidophilus* in the carrier solution was 9.02 ± 0.02 log cfu/g.

2.4. Spray drying and storage

Carrier media containing *L. acidophilus* NCIMB 701748 were dried using a laboratory spray dryer (Buchi B-290, Flawil, Switzerland) operated at constant inlet temperature of 134 ± 1 °C, feed flow rate of 7.14 mL/min, drying air flow rate of 35 m^3/h, and compressor air pressure of 0.5 MPa, as previously reported by Behboudi-Jobbehdar et al. (2013). The outlet temperature achieved was 76 ± 2 °C and the carrier solution was constantly stirred throughout the spray drying process using a magnetic stirrer. The dry probiotic formulations were collected from the cyclone, placed in sealed glass vials and stored in desiccators containing saturated LiCl solution (a_w = 0.11), inside an incubator at 25 °C. The entire process for the production of the probiotic powders was performed in duplicate and samples from both batches were used for the technological, microbiological and *in vitro* digestion analyses.

2.5. Enumeration of viable bacteria

The samples (spray dried powders or drying media) were suspended in PBS and mixed by mechanical shaking for 10 min at room temperature to ensure complete dissolution of the powders. Serial dilutions were prepared from the initial suspension and pour plated on MRS agar (MRS Agar, Oxoid Ltd., Basingstoke, UK), in duplicate. Plates were incubated at 37 °C for 72 h under anaerobic conditions and the number of colonies were counted (Champagne et al., 2011). Results are expressed as log colony forming units per gram (log cfu/g).

The *L. acidophilus* NCIMB 701748 inactivation upon storage was expressed as the logarithmic value of the relative viability fraction ($\log N/N_0$). The viability data were fitted to a first order reaction kinetics model as described by Eq. (1).

$$\log N_t = \log N_0 + k_T t \quad (1)$$

where N_0, represents the initial number of the viable bacteria (cfu/g), and N_t the number of viable bacteria after a specific time of storage (cfu/g), t is the storage time (day), and k_T is the inactivation rate constant at temperature T (day^{-1}).

2.6. Survival of encapsulated bacteria during in vitro digestion

Spray dried powders and free bacteria were compared for their ability to survive *in vitro* digestion simulating the human gastric and intestinal environments. The method was based on a previously published procedure (Yonekura & Nagao, 2009) with modifications to better resemble the gastric pepsin activity (1600 unit/mL) and duodenal bile salt concentration

Table 1 – Composition of drying media.

	Control	Sodium alginate	Chitosan	HPMC
Maltodextrin (g)	12	12	12	12
Glucose (g)	4	4	4	4
Whey protein concentrate (g)	3	3	3	3
Sodium alginate (g)		5		
HPMC (g)				5
Chitosan (g)			0.5	
Lactic acid (g)			0.5	
Distilled water (g)	81	76	80	76
Total solids (%)	19	24	19.5	24
pH	6.39	6.42	3.89	6.34

(4.4 g/L) found in human aspirates (Faye, Tamburello, Vegarud, & Skeie, 2012; Reppas & Vertzoni, 2012). Three-hundred milligrams of spray dried powders or the equivalent counts of viable bacteria (pelleted from MRS broth and washed once with PBS) were suspended in 2.5 mL saline in 30 mL glass vials, then 2.5 mL of double-concentrated simulated gastric juice was added (pH 2.5, 3200 unit/mL pepsin, 7.2 mmol/L $CaCl_2$, 3 mmol/L $MgCl_2$, 98 mmol/L NaCl, 24 mmol/L KCl, and 12.8 mmol/L KH_2PO_4). The vials were screw-capped under a stream of N_2 and incubated for 1 h at 37 °C and constant magnetic stirring at 130 rpm/min (gastric digestion). Pancreatin and bile salts were dissolved in 0.1 mol/L $NaHCO_3$, added to the gastric digesta (final concentrations were 4.4 g/L bile salts and 4 mg bile salts), and incubated for 2 h. Aliquots of the digesta were diluted in PBS and plated on MRS agar for enumeration of viable bacteria, as described above. Survival ratios were calculated as the log-transformed ratio between viable counts per vial before and after the simulated digestion ($\log N/N_0$).

2.7. Characterisation of spray dried powders

2.7.1. Residual water content and water activity

The residual water content was calculated according to AACC method 44-15.02 (AACC International, 2001). Briefly, 2 g samples of the powder formulations were placed in aluminium pans, dried at 105 °C for 24 h, and the residual moisture was expressed as percentage of the initial weight.

Water activity of the powders after spray drying was measured using an AquaLab water activity meter (AquaLab, 3TE, Decagon, USA).

2.7.2. Differential scanning calorimetry

A standard power compensated Perkin Elmer DSC-7 (Perkin Elmer Ltd., Beaconsfield, UK) was used for the determination of glass transition temperature of the probiotic formulations, as described by Behboudi-Jobbehdar et al. (2013). A small sample (10–20 mg) of the powder was weighed in a stainless steel DSC pan and heated from −30 to 150 °C at the rate of 10 °C/min. A double heating–cooling scanning step was performed, and the onset and midpoint glass transition temperatures were calculated using the Mettler Toledo Star software from the second heating step thermographs.

2.7.3. Particle size analysis

Particle size analysis was performed on a laser diffraction particle size analyser equipped with Tornado dry powder system (LS 13 320, Beckman Coulter, USA). The Fraunhofer theory was used for the determination of the mean diameters of the microcapsules (Soukoulis et al., 2013).

2.7.4. Hygroscopicity

The hygroscopicity of the probiotic powders (1 g) was determined gravimetrically as percentage of weight increase over a 5 days equilibrium period (75% RH desiccator chamber containing saturated NaCl) at room temperature (Fritzen-Freire et al., 2012).

2.7.5. Morphological characterisation by scanning electron microscopy (SEM)

A small amount of powder was carefully deposited onto carbon tabs (Agar Scientific, Stansted, UK), coated with carbon (Agar turbo carbon coater) and placed on the stage of a FEI Quanta 3D 200 dual beam Focused Ion Beam Scanning Electron Microscope (FIB-SEM; FEI, Hillsboro, USA). Images were acquired using secondary electron imaging at an accelerating voltage of 5–15 kV.

2.8. Statistical analysis

Factorial ANOVA followed by Duncan's post hoc means comparison ($p < 0.05$) test was performed to evaluate the effects of soluble fibres and processing/storage (spray-drying and 5 week storage at room temperature) on the viability of *L. acidophilus* NCIMB 701748. One-way ANOVA and Duncan's post hoc comparison were used to evaluate the effect of soluble fibres on bacterial viability after *in vitro* digestion. All statistical calculations were performed using MINITAB release 16 (Minitab Inc., PA, USA).

3. Results and discussion

3.1. Impact of soluble dietary fibre on *L. acidophilus* NCIMB 701748 viability upon spray drying

There was no difference in the starting counts of viable bacteria across all carrier media. Total counts ranged from 8.90 to 8.99 log cfu/g of solids (Fig 1). This indicates that there was no acute toxic effect of the fibres on *L. acidophilus* NCIMB 701748 whilst in aqueous media.

The addition of sodium alginate and HPMC to the drying media maintained the viability of *L. acidophilus* NCIMB 701748 in spray dried powders, no significant differences were observed between the cell counts in fibre-free control (8.40 log cfu/g), sodium alginate (7.96 log cfu/g) and HPMC (8.73 log cfu/g) powders (Fig 1). However, the addition of chitosan resulted in a reduced process stability for *L. acidophilus* NCIMB 701748 during drying, resulting in powders with lower viable count (5.312 log cfu/g) when compared to the control.

Considering that both the control samples and the samples containing fibre were formulated with the thermoprotective ingredients whey protein concentrate and D-glucose at fixed concentrations and spray drying was performed in optimized conditions for *L. acidophilus* NCIMB 701748 (Behboudi-Jobbehdar et al., 2013), the control system already had a high survival rate after spray drying: 29.9% (8.93–8.40 log cfu/g). Desmond et al. (2002) reported an improvement of the viability of *L. paracasei* NFBC338 encapsulated after adaptation and drying in reconstituted skim milk with gum acacia, however the improvement was from less than 0.01% to 0.9 ± 1.3% and only at an outlet temperature of 100–105 °C whilst no effect of gum acacia was observed at the lower outlet temperature of 95–100 °C in the same study. Therefore, as both the composition of control carrier medium (maltodextrin, whey protein concentrate and glucose) and the spray drying conditions have been previously optimized for *L. acidophilus* NCIMB 701748 (Behboudi-Jobbehdar et al., 2013; Soukoulis et al., 2013), and relatively mild outlet temperatures (76 ± 2 °C) were kept during drying, it is possible that the survival rate in control medium had already reached near maximum values for this *L. acidophilus* strain. in line with our results.

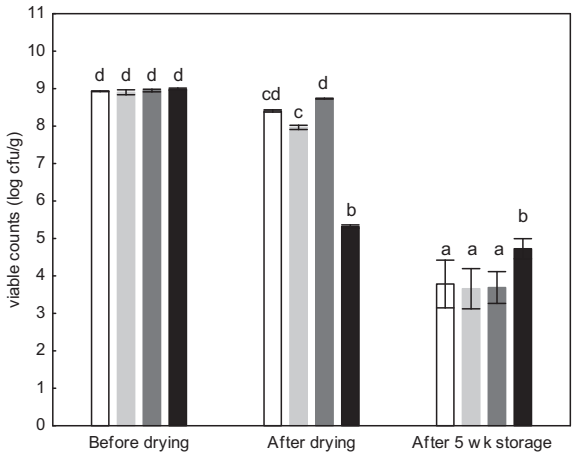

Fig. 1 – Total viable count (log cfu/g) of *L. acidophilus* NCIMB 701748 before and after spray drying, and at the end of 5 weeks of storage at room temperature. Bars denote mean ± standard deviation of viable counts from control (empty bars), sodium alginate (light grey), HPMC (dark grey) and chitosan (black) drying media (per gram of total solids) or spray dried powders. Factorial ANOVA detected a significant effect of fibres ($p < 0.01$) and processing/storage ($p < 0.001$) on viable counts of bacteria. Bars labelled with different letters are significantly different ($p < 0.05$) by Duncan's post hoc comparisons.

Corcoran et al. (2004) reported that addition of inulin did not improve viability during spray-drying of *L. rhamnosus* GG at outlet temperatures of 85–90 °C. Bustos and Bórquez (2013) have also shown no improvement of *L. plantarum* viability after spray drying through the addition of arabic gum or pectin, when compared to whey proteins alone.

The greater loss of viability observed in the chitosan system may be explained by the positively charged amino groups of chitosan, which have been previously reported to exert antimicrobial activity, interacting with the polyanionic components of cell walls (Kong, Chen, Xing, & Park, 2010); this interaction, in combination with the additional stress of spray drying may have triggered the loss of cell viability.

3.2. Storage stability of *L. acidophilus* in spray-dried powders at 25 °C

The type of the soluble dietary fibre significantly influenced the viability of *L. acidophilus* NCIMB 701748 over storage, with reduction of its viable counts ranging from 0.27 to 4.5 log cfu/g. Chitosan addition significantly enhanced storage stability of spray dried powders as shown by the remarkably lower inactivation rate (0.007 ± 0.001 day^{-1}) compared to all other systems (0.114–0.141 day^{-1}, Table 2, Fig. 2) and significantly higher viable counts in chitosan powders (4.72 log cfu/g) compared to all other systems (3.66–3.78 log cfu/g, Fig. 1) at the end of the 35-day storage, in spite of the lower initial counts. Inactivation rates of HPMC were slightly higher and that of sodium alginate was slightly lower than the control (Table 2), however the differences were small and did not affect the viable counts after 35 days of storage (Fig. 1).

Lapsiri et al. (2012) have reported similar inactivation rates for *L. plantarum* TISTR 2075 in spray dried powders based on maltodextrin, but none of the putative protective ingredients (trehalose, soy protein isolate, Fibersol® and gum acacia) were able to improve inactivation rates over storage at 25 °C. In the case of skimmed milk-based growth/drying media, partial substitution of skim milk by gum acacia improved storage stability of spray-dried *L. paracasei* NFBC 338 (Desmond et al., 2002) whilst the use of polydextrose or inulin had no effect on the stability of *L. rhamnosus* GG (Corcoran et al., 2004). Therefore, our results and literature data agree that soluble fibres cannot be considered on their own as thermoprotective agents or shelf life enhancers, and such effects are likely to be a result from a range of properties of the spray-dried microcapsules.

It is well established that the synergism between the physical state of the matrix and the storage temperature critically affect the viability of spray dried probiotics (Ananta et al., 2005; Fu & Chen, 2011; Meng, Stanton, Fitzgerald, Daly, & Ross, 2008). Most developed spray dried probiotic systems are able to maintain viability at ~4 °C, but the real challenge is to ensure a reasonable shelf life for these products at room temperature. As the viable counts in chitosan-containing powders did not show a significant decrease even after 35 days of storage at 25 °C, it indicates a possible application of chitosan as a shelf-life enhancer of anhydrobiotics, provided its negative effect during spray drying is overcome. The relationship between the physical characteristics of spray dried particles and cell viability over storage is further discussed towards the end of this article.

3.3. Survival of microencapsulated bacteria in GI simulated conditions

To assess the effect of spray drying on the survival of bacteria during simulated digestion, a sample of free bacteria harvested from MRS broth and re-suspended in saline was run in parallel with the spray dried powders. The initial load of bacteria ranged from 3.81 to 5.43 log cfu according to viable counts in each formulation as the amount of powder per vial was kept constant (0.3 g), thus the cell survival is expressed as log-transformed ratios (log N/N_0, and is shown in Fig. 3). Survival ratios of *L. acidophilus* NCIMB 701748 in chitosan matrices were significantly higher than the control and free bacteria (Fig. 3). On the other hand, HPMC and sodium alginate systems had a worse performance during the simulated digestion, with survival ratios lower than the control and free bacteria (Fig. 3).

The fibre-free control powders maintained the same survival ratio as the free bacteria that had not been subjected to spray drying, showing that cells surviving the drying process did not have heat induced sub-lethal injuries. In practical terms, the control carrier which was formulated with the thermoprotective ingredients (maltodextrin, glucose and whey protein concentrate) and dried at relatively low outlet temperatures (76 ± 2 °C) resulted in powders containing viable cells with minor or no injuries, which were able to withstand the simulated gastrointestinal conditions equally as well as free cells freshly harvested from MRS broth. Heat-injured cells are more prone to inactivation in harsh environments

Table 2 – Physical and thermophysical properties of L. acidophilus NCIMB 701748 spray-dried powders containing different soluble dietary fibres and their inactivation rates throughout 35-day storage at 25 °C. Data is expressed as mean ± standard deviation. Residual water content is expressed as g/100 g of product; hygroscopicity is recorded at 75% over 5 days.

	Control	HPMC	Sodium alginate	Chitosan
a_w	0.25 ± 0.01[a]	0.29 ± 0.01[b]	0.25 ± 0.01[a]	0.28 ± 0.02[a,b]
Residual water content (g/100 g)	1.89 ± 0.07[a]	2.40 ± 0.10[b]	1.86 ± 0.17[a]	3.96 ± 0.09[c]
Hygroscopicity (g/100 g)	11.63 ± 0.53[a]	13.65 ± 0.62[b]	11.44 ± 0.55[a]	15.73 ± 0.81[c]
Particles mean size, $d_{V,50}$ (μm)	13.46 ± 0.14[b]	12.09 ± 0.07[a]	14.73 ± 0.06[d]	13.78 ± 0.07[c]
T_g onset (°C)	26.0 ± 0.2[c]	24.4 ± 0.4[a]	33.5 ± 0.3[d]	27.1 ± 0.4[b]
T_g midpoint (°C)	45.9 ± 0.4[c]	41.9 ± 0.1[b]	50.9 ± 0.4[d]	39.9 ± 0.9[a]
Specific heat change, ΔC_p (kJ/mol K)	0.454 ± 0.02[d]	0.337 ± 0.02[b]	0.376 ± 0.06[c]	0.284 ± 0.04[a]
Inactivation rate, k_t (day^{-1}) (R^2 value of inactivation curve)	0.123 ± 0.002[c] (0.939)	0.141 ± 0.003[d] (0.984)	0.114 ± 0.002[b] (0.936)	0.007 ± 0.001[a] (0.810)

[a] Different letters within individual rows indicate a significant difference according to Duncan's means post hoc comparison test.

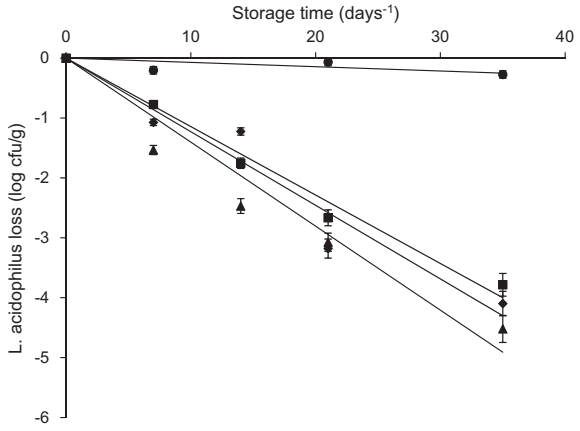

Fig. 2 – Viability loss of *L. acidophilus* NCIMB 701748 in spray-dried powders during storage at 25 °C; ♦ = control, ■ = sodium alginate, ▲ = HPMC, ● = chitosan.

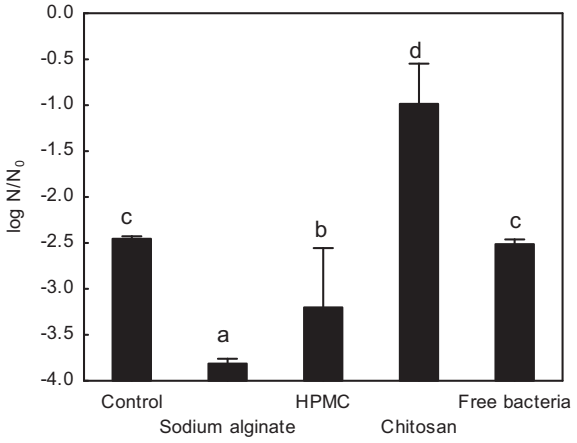

Fig. 3 – Survival of free and encapsulated *L. acidophilus* NCIMB 701748 after *in vitro* digestion. Bars express the log-transformed ratio of bacterial counts before and after the simulated digestion (mean ± standard deviation). At the start of *in vitro* digestion reaction vials contained: control 5.43 ± 0.03 log cfu, sodium alginate 3.63 ± 0.05 log cfu, HPMC 5.01 ± 0.64 log cfu, chitosan 3.81 ± 0.44 log cfu and free bacteria 5.12 ± 0.05 log cfu digestion (mean ± standard deviation). Bars labelled with different letters are significantly different ($p < 0.05$) by Duncan's post hoc comparisons.

such as high salt (Corcoran et al., 2004) and gastrointestinal conditions.

Although spray drying of chitosan-containing carriers resulted in large losses of viable bacteria, cells that were effectively encapsulated and survived the drying process not only remained viable but also had additional protection from chitosan against simulated gastrointestinal conditions. The additional protective effect on *L. acidophilus* NCIMB 701748 viability may be due to the low solubility of chitosan (it only dissolves with addition of acid) that may have resulted in a slower rehydration of the powder and release of the encapsulated bacterial cells. A slow rehydration may improve cell viability, by slowing down the influx of water through cell membranes, especially at critical times during the phase transitions of lipid bilayers (Poirier, Maréchal, Richard, & Gervais, 1999; Teixeira, Castro, & Kirby, 1995). Furthermore chitosan may also offer a localised buffering effect (Cook, Tzortzis, Charalampopoulos, & Khutoryanskiy, 2012). Cook et al. (2011) have demonstrated that an outer layer of chitosan added to alginate beads delayed the release of probiotic cells from dry microbeads during gastric digestion, and enhanced the viability during digestion in simulated intestinal solutions. Chitosan has also been reported to inhibit pancreatic lipase activity (Tsujita et al., 2007), which may have protected the *L. acidophilus* cell membrane against hydrolysis resulting in higher cell viability in our simulated GI conditions. This effect on lipase seems to be mediated by the positively charged functional groups (Tsujita et al., 2007) in the chitosan molecule, a feature that is not shared by other fibres tested in our study.

Another feature of the carrier media containing chitosan is the lower pH induced by the addition of lactic acid, which is added to promote adequate dissolution of chitosan. Although most lactic acid bacteria develop acid tolerance when subjected to sub-lethal acidic environments during the growth stage (Lorca, Raya, Taranto, & de Valdez, 1998; van de Guchte et al., 2002), the *L. acidophilus* culture used was in the stationary stage when pelleted and suspended in the carrier solution with chitosan. Therefore whist enhanced acid tolerance maybe a reason for enhanced gastric stability we cannot assume that

increased acid tolerance is the underlying mechanism for increased survival of *L. acidophilus* NCIMB 701748 in chitosan-containing spray-dried powders during simulated digestion as the culture is in the stationary phase during preparation.

On the poor survival of *L. acidophilus* NCIMB 701748 in powders containing sodium alginate and HPMC during simulated digestion, cells in these formulations may have suffered sub-lethal heat injuries. Such injuries would not reflect on the viable counts after spray drying but lower the resistance of the cells in harsh environments such as those in the simulated digestion (e.g. bile salts, enzymes) (Golowczyc, Silva, Teixeira, De Antoni, & Abraham, 2011). Similarly, Corcoran et al. (2004) reported that addition of inulin to skim milk-based drying medium resulted in more damage to *Lactobacillus Rhamnosus* GG during spray drying, as shown by their higher sensitivity to 5% NaCl after drying.

3.4. *Characterisation of spray-dried powders*

3.4.1. *Physical properties*

The water activity of the powders ranged from 0.25 to 0.29 with sodium alginate having the highest a_w value although the range of differences was relatively small (Table 2). Control and sodium alginate samples (1.86% and 1.89% w/w) had lower residual water content values compared to HPMC(2.40% w/w) and chitosan (Table 2, 396% w/w). However, in all cases the residual water content was lower than 4%, a generic value for the maximum acceptable levels for food powders (Ananta et al., 2005). Parameters such the hygroscopicity and the water binding ability of the matrix components can affect the water activity and residual water contents in food powders (Barbosa-Canovas, Ortega-Rivas, Juliano, & Yan, 2005), in line with our results where samples with the lowest moisture contents (control and sodium alginate) had the lowest hygroscopicity values.

3.4.2. *Thermophysical properties*

Amorphous materials such as spray dried powders are generally characterised by their ability to undergo transitions from a highly viscous (glassy) state to rubbery state. This phase transition is typically accompanied by a significant decrease of the viscosity and an increase of the plasticiser's molecular mobility leading to powder destabilisation, e.g. agglomeration, caking, stickiness, reduced flowing ability, acceleration of enzymatic and chemical reaction rates (Meste, Champion, Roudaut, Blond, & Simatos, 2002). In the case of anhydrobiotics (dried probiotics), the physical state of the matrix is not only related with its storage stability in terms of techno-functional properties but also with the retention of bacterial cell viability throughout the drying process and upon storage (Fu & Chen, 2011).

Apart from sodium alginate based systems, the rubbery to glassy state transition (T_{on}) was found to be initiated close to room temperature (Table 2) and 244 °C for HPMC and 27.1 °C for chitosan based systems). The glass transition temperatures ranged from 39.9 °C to 50.9 °C (chitosan and sodium alginate containing systems, respectively) with the control exhibiting an intermediate glass transition temperature. However, as was expected, the change in the specific heat (ΔC_p) during the glassy to rubbery state transition was significantly lower for all the soluble dietary fibre containing systems compared to control (Table 2), implying that addition of fibre further lowers the water molecule's mobility. For a spray dried powder the plasticiser content and the thermophysical properties of the individual components of the matrix are among the most important factors that drive its thermophysical profile. In our system, water is the main plasticiser but low molecular weight carbohydrates, i.e. D-glucose and lactose, may also act as secondary plasticising agents. Indeed, a significant linear correlation ($p < 0.001$, $r = 0.926$) was found between the T_g and the residual water content of the spray dried powders. However, the differences in the glass transition temperature between the control and sodium alginate systems (which had similar residual water content) could be attributed to the higher ratio of D-glucose and lactose to total solids (0.279 g/g for control and 0.211 g/g for sodium alginate). On the other hand, the reduction of ΔC_p values in the presence of polysaccharides indicates small changes in the molecular mobility of water during the glassy to rubbery state transition, this is well established in the case of systems containing macromolecular hydrocolloids such as proteins or polysaccharides (Mainville, Arcand, & Farnworth, 2005).

3.4.3. *Particle size analysis and morphological characterisation*

The HPMC based probiotic formulations had a significantly ($p < 0.01$) smaller mean particle size diameter compared to the control (Table 2), and sodium alginate and chitosan formulations had a greater mean particle size than the control, although the differences are very small. Many parameters have been reported to influence the particle size of spray dried food powders including the drying process; the viscosity of the drying media; the presence of surface active components; the total solids of the drying medium; the atomizer type and the nozzle diameter (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). In our series of experiments all the processing conditions were kept constant and consequently viscosity or surface tension of the drying liquid appear to be impacting particle formation. Carneiro, Tonon, Grosso, and Hubinger (2013), reported that that higher carrier medium viscosity resulted in larger droplets being formed during atomization and consequently larger particles after spray drying. Furthermore surface active proteins or polysaccharides can promote the formation of smaller liquid droplets during atomization, due to their ability to stabilise air–water interfaces, film forming abilities and amphiphilic chemistry (Gharsallaoui et al., 2007). In contrast to sodium alginate and chitosan, HPMC is well known for its ability to lower surface tension and adsorb onto air–water interfaces (interfacial tension, at 25 °C, of water, sodium alginate, chitosan and HPMC is 72, 63–68, 70–72 and 41–43 mN/m, respectively (Lee, Chan, Ravindra, & Khan, 2012; Qun & Ajun, 2006). Our results therefore imply that the powder mean size may have been influenced synergistically both by the carrier solution viscosity and the amphiphilic character of the associated hydrocolloids. Indeed, the use of dietary fibres with high viscosities such as sodium alginate led to the highest particles mean size whilst HPMC, which has similar viscosities to the control (informally measured during formulation) and higher surface activity, favoured the formation of smaller droplets in the atomiser.

From a microstructure perspective, all powders displayed a partially collapsed spherical shape with concavities (Fig. 4).

According to Fig. 4, there was no evidence of surface fissures or rupture of the microspheres which confirms good structural integrity and suggests low gas permeability e.g. oxygen, water vapour (Fritzen-Freire et al., 2012). In addition, the absence of free surface bacteria indicates good encapsulating performance of the powders. The microstructural aspects of spray dried products are critically influenced by several parameters such as the drying rates, composition, viscosity of the drying carrier aliquots and the atomisation process (Kim, Chen, & Pearce, 2009). It has been reported that the implementation of intermediate drying rates during spray drying promotes the partial collapse of the particles (Rodriguez-Huezo et al., 2007). Compared to the control samples, the presence of polysaccharides reduced the collapse of the microcapsules but at the same time led to the formation of shrivelled spherical particles which was particularly evident in the case of sodium alginate and chitosan systems. Kim et al. (2009) previously reported that an increase of feed solids caused a significant restriction of water and solutes migration within viscous droplets drying and thus led to the formation of bigger particles with less evidence of structural collapse. Moreover, the presence of surface active compounds would favour migration to the air–water interface potentially indicating a spatial distribution of surface active material across the powder.

3.5. *Physical characteristics of spray-dried powders and their influence on storage stability of L. acidophilus in spray-dried powders at 25 °C*

Recently, we have investigated the storage stability of *L. acidophilus* NCIMB 701748 produced in optimal drying conditions, in different matrix compositions, physical states, and storage at 4 °C, 25 °C and 35 °C (Soukoulis et al., 2013). The highest performance was achieved in glassy matrices containing whey protein concentrate and D-glucose stored at 4 °C, and therefore this carrier has been chosen as our control. In the present study, inactivation rates of *L. acidophilus* NCIMB 701748 in powders stored at 25 °C were in the following order: HPMC > control > alginate > > > chitosan. To explain the possible mechanisms underlying the post-drying inactivation of *L. acidophilus* NCIMB 701748 shown herein, correlations between the physicochemical properties and inactivation rates of *L. acidophilus* NCIMB 701748 were tested. Regressions (Pearson's) using the entire data set were inconclusive mainly due to the peculiar behaviour of chitosan-containing systems during both spray drying and storage. By removing chitosan from the dataset, k_T was highly ($p < 0.05$) correlated with $T_{g,onset}$ ($r = -0.853$), T_g ($r = -0.968$), a_w ($r = 0.945$), RWC ($r = 0.968$) and viability after spray drying ($r = -0.999$). Based on the observed correlations we suggest two possible mechanisms to explain the functionality of HPMC and alginate as encapsulants. Firstly, that related to the thermal damage of the cell membrane during spray drying and secondly that related to the changes in the amount of bound water and the molecular mobility of water within the spray dried powder. Indeed, Gardiner et al. (2000) observed that the heating during the drying process causes non-adverse structural changes of the probiotic cells which influence not only the post-drying viability but also their sensitivity to storage conditions (demonstrated by NaCl tolerance testing).

On the other hand, changes in the mobility of water molecules are also very important, particularly in the case of physical states controlled by WLF kinetics (rubbery). All systems, except for sodium alginate, were found in a physical state that was close to WLF kinetics at the selected storage temperature (T_{ref}, 25 °C) as is indicated by the small

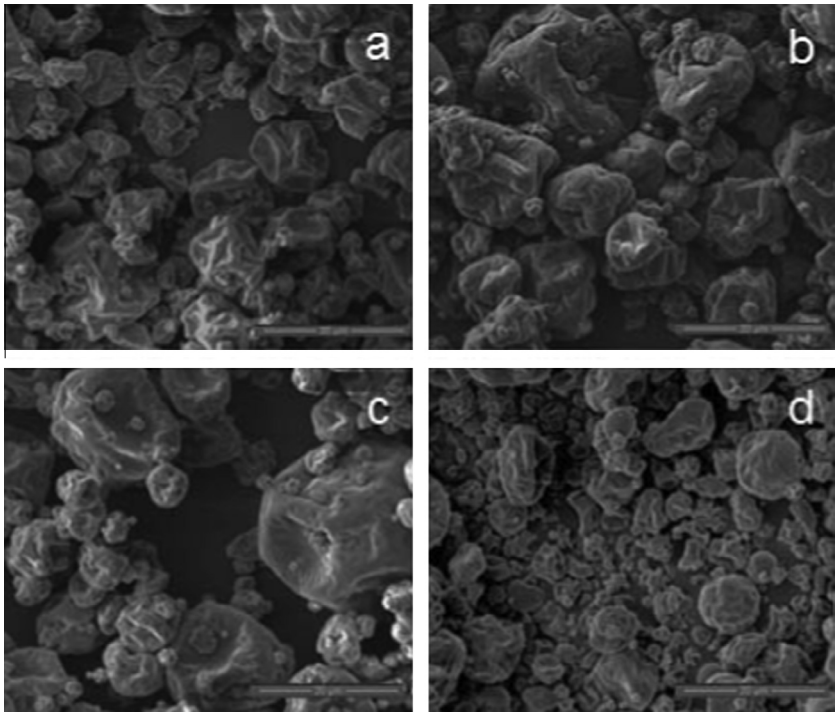

Fig. 4 – SEM micrographs of *L. acidophilus* NCIMB 701748 spray-dried microcapsules containing different soluble dietary fibres. a = control, b = HPMC, c = sodium alginate, d = chitosan. Bars in each image are equivalent to 20 μm.

differences between the $T_{g,onset}$ and T_{ref} values (−0.6 °C for HPMC and 1.0 °C for control). This practically suggests that water mobility in the control and the HPMC powder is higher compared to sodium alginate systems ($T_{ref}-T_{g,onset}$ = −8.5 °C), increasing the availability of water molecules for potentially lethal biochemical reactions. On the other hand, *L. acidophillus* NCIMB 701748 in chitosan-containing systems showed the best storage stability despite its comparable physical state to the control and HPMC systems (T_{ref}–$T_{g,onset}$ = 2.1 °C). Although there is no clear explanation of the excellent performance of the chitosan systems during storage, we speculate that the ability of chitosan to gel on the surface of droplets during the drying process increases the polymer concentration at the exterior of the dried microcapsules (Cook et al., 2011), raising a barrier against toxic environmental factors such as water vapour and oxygen. The latter could also find support in the longer time required for complete dissolution indicating lower diffusion rates of water from the bulk to the liquid–solid interface. An improved viability of *Lactobacillus casei* in dried alginate micro beads in the presence of chitosan and carboxymethylated chitosan has been also reported by Li et al. (2011) and supports this hypothesis.

4. Conclusions

The microencapsulation of probiotics enables storage of viable bacteria at room temperature and may allow incorporation of probiotics into a wide range of food products without imparting undesirable sensory characteristics. While investigating the effect of additional soluble fibres in carriers for spray drying of *L. acidophilus* NCIMB 701748 we showed that although chitosan causes large losses of viability during spray-drying, these losses are counteracted by the excellent stability over storage at room temperature and the overall effect becomes positive after 5 weeks of storage. Chitosan also improved survival rates in simulated gastrointestinal conditions, however no single fibre could improve *L. acidophilus* NCIMB 701748 viability in all steps from production through storage and digestion. It is anticipated that tailored composite carriers would have to be developed for commercial applications and that the information on differential stability by each carrier would be a valuable tool to aid decision making in this area.

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