Carbohydrate Polymers 102 (2014) 423–430

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Protection of *L. rhamnosus* by spray-drying using two prebiotics colloids to enhance the viability

Sandra V. Avila-Reyes[a], Francisco J. Garcia-Suarez[a], María Teresa Jiménez[b], María F. San Martín-Gonzalez[c], Luis A. Bello-Perez[a,*]

[a] Centro de Desarrollo de Productos Bióticos (CEPROBI) del Instituto Politécnico Nacional, Yautepec, Morelos, Mexico
[b] Departamento de Ingeniería Química, Alimentos y Ambiental, Universidad de las Américas Puebla, Mexico
[c] Department of Food Science, Purdue University, West Lafayette, IN 47907, United States

ARTICLE INFO

Article history:
Received 16 April 2013
Received in revised form
11 November 2013
Accepted 20 November 2013
Available online 4 December 2013

Keywords:
Starch
L. rhamnosus
Viability
Protection
Spray-drying

ABSTRACT

Protection of probiotics by substances considered as prebiotics can be an alternative to increase their viability in the large intestine. The objective of this study was to use two wall materials (native rice starch and inulin) without bonding agent to protect *Lactobacillus rhamnosus* during spray-drying and determine the viability of the microorganism under two storage conditions. For spray-drying conditions tested in this work the product yield with native rice starch (NRS) ranged between 65% and 74% whereas for inulin (IN) it ranged between 43% and 54%. In general, IN solutions exhibited higher outlet temperature than NRS dispersions. Capsules of IN had smaller particle size than those of NRS. Due to the higher hydrophilic nature of IN capsules as compared to NRS, IN capsules exhibited higher water activity than NRS capsules. Confocal microscopy showed marked differences between both wall materials, which could in turn cause differences in the release profile of encapsulated microorganisms. Agglomerates of NRS provided better protection to the microorganisms as evidenced by the lower reduction in viability when compared to IN, and this effect was corroborated by the stability study. It is possible to protect probiotics using both colloids, but differences in the viability and stability during storage were determined. The use of IN could prove beneficial in the encapsulation of probiotic strains since this carbohydrate is not hydrolyzed by human digestive enzymes and may act as prebiotic.

1. Introduction

Microencapsulation (ME) is a process used to protect active ingredients against adverse environmental factors and to improve the delivery of bioactive molecules and living cells into foods (Nedovica, Kalusevic, Manojlovic, Levic, & Bugarski, 2011) by enclosing the active ingredients within a carrier material. The production of functional foods containing encapsulated probiotic bacteria provides health benefits to the consumers, is an area currently under extensive research. In particular, the range of dairy products incorporating probiotics has experienced a tremendous growth. A major challenge faced by the product developer is to ensure that probiotic microorganisms withstand the harsh environments and stresses encountered during processing, storage and transit through the gastrointestinal tract, so that they are delivered in sufficient numbers for conferring the health benefits expected from them (Burgain, Gaiani, Linder, & Scher, 2011). Spray-drying of bacterial dispersions in protective colloid solutions is a convenient means for manufacturing powders harboring high numbers of viable microorganisms that could facilitate the handling and storage of probiotic cultures and their subsequent application in functional foods (Rodríguez-Huezo et al., 2007). However, to ensure the economic feasibility of the spray-drying process, the initial dispersions should have a relative high concentration (~25–40 wt%) and possess a relatively low viscosity. These restrictions imposed by the process limit the choice of protective colloids available in the market that comply with one or both of the aforementioned requirements. Among available colloids the use of gum Arabic (Desmond, Ross, O'Callaghan, Fitzgerald, & Stanton, 2002), starch or modified starches (Goderska & Czarnecki, 2008; O'Riordan, Andrews, Buckle, & Conway, 2001); whey protein (Picot & Lacroix, 2004), maltodextrins (Boza, Barbin, & Scamparini, 2004), cellulose (Favaro-Trindade & Grosso, 2002) or combinations of some these hydrocolloids or others (Chavez & Ledeboer, 2007; Rodríguez-Huezo et al., 2007) have been investigated as wall materials during spray-drying process. The main disadvantage of spray-drying technique is the high temperature used in the process causes simultaneous dehydration and thermal inactivation of probiotic cells during the drying process (Anal & Singh, 2007; Ivanovska et al., 2012). Proper adjustment and control of processing conditions such as inlet and outlet air temperatures are very important for achieving viable encapsulated cultures of

* Corresponding author. Tel.: +52 735 3942020; fax: +52 735 3941896.
E-mail address: labellop@ipn.mx (L.A. Bello-Perez).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.11.033

desired particle size distribution (Kailasapathy, 2002). Likewise, spray-drying restricts the selection of probiotics to those strains that can better adapt to the stresses induced during processing, which in turn increases the probability of surviving under harsh conditions during storage or digestion of the foods to which they are added (Nazzaro, Orlando, Fratianni, & Coppola, 2012).

One of the main challenges in the ME of probiotics is to find effective encapsulating materials that preserve the viability of the culture; in this regard, there is increasing interest in the use of probiotic/prebiotic combination (named "synbiotic") due to that when probiotics reach to colon, they could use the prebiotics for survival and implantation that beneficially affect the host (Figueroa-Gonzalez, Quijano, Ramırez, & Cruz-Guerrero, 2011; Nazzaro et al., 2012; Rokka & Rantamäki, 2010). Different types of starch, including modified starches, from diverse botanical sources have been used to protect probiotics (Crittenden et al., 2001). Native starches with small granule size, e.g. native rice starch, contain high levels of slowly digestible starch (SDS) and resistant starch (RS) (Zhang, Xie, Shi, & Xue, 2006); RS is considered as prebiotic (Fuentes-Zaragoza et al., 2011). Hydrothermal treatments with different moisture content of native rice starch increased the RS content (Da Rosa Zavareze et al., 2012), starch-chain associations contributed to this change (Puncha-Arnon & Uttapap, 2013). Upon spray-drying small granule native starches form spherical aggregates where probiotics are entrapped (Zhao & Whistler, 1994). Since digestive enzymes do not hydrolyze RS, the microencapsulated probiotics reach the large intestine where the microbiota hydrolyze the starch granules and release the probiotics in the gut, thus increasing or restoring the microbiota. Additionally, short-chain fatty acids (SCFA), which also contribute to restoring or increasing the microbiota, are produced during fermentation of the prebiotics. Inulin is a blend of fructose polymers and is classified as storage carbohydrate present in crops such as onions, garlic, Jerusalem artichoke, chicory and agave tequilana (Figueroa-Gonzalez et al., 2011; Kalyani Nair, Kharb, & Thompkinson, 2010). Inulin is not hydrolyzed by digestive enzymes and it is fermented in the colon by the microbiota, for this reason is considered as prebiotic and has been added along with probiotics in the manufacture of nutritive bars (Nazzaro et al., 2012). The aim of this study was to use native rice starch and inulin to encapsulate *Lactobacillus rhamnosus* by spray-drying and evaluate the viability of the microorganism during storage.

2. Materials and methods

2.1. Materials

Native rice starch (NRS) was isolated as previously reported (Palma-Rodriguez et al., 2012), whereas chicory inulin (*Cichorium intybus* L.) (IN) (Beneo™ ST, Orafti) was a gift from Megafarma Feed. Ascorbic acid was purchased from Fermont (Monterrey, Mexico).

The probiotic bacteria *L. rhamnosus* B442 was obtained from the collection of Food Microbiology Laboratory of the University of the Americas Puebla, México, and was produced by fermentation using MRS agar (Man–Rogosa–Sharpe), kept at refrigeration temperature, which was reactivated by placing in liquid MRS broth (B.D. Difco™, México), and it is re-growing every 2 months to keep the viability.

2.2. Preparation of microencapsulated *L. rhamnosus* B442 powders

The formulations used for the production of *L. rhamnosus* B442 spray-dried microcapsule powders are shown in Table 1. Both wall materials (NRS or IN) were dispersed in sterile water at room temperature at concentration showed in Table 1 to obtain 400 g. The mixtures were maintained under constant agitation for 30 min at 25 °C.

2.3. Density

The density of the various mixtures was determinate by the AOAC 962.37 method (AOAC, 1995). A glass pycnometer (previously equilibrated to constant weight at 25 °C) was used. The density was calculated using the following equation:

$$\rho_s = \frac{(m_3 - m_1)}{(m_2 - m_1) \times \rho H_2O}$$

where ρ_s is the density of solution (g/cm^3), m_1 is the mass of empty pycnometer (g), m_2 is the mass of pycnometer with water (g), m_3 is the mass of pycnometer with dispersion (g), and ρ_{H_2O} is the density of water.

2.4. Viscosity

The viscosity of the mixtures was determined using a Cannon-Fenske viscometer (Mod. 350-152-1, Cannon Instrument Company, PA, USA) (Geankoplis, 1998).

2.5. Growth curve of *L. rhamnosus*

The growth curve of *L. rhamnosus* was performed to corroborate its growing stage and determine the time when the microorganism should be mixed with the wall material. The microorganism was cultured initially on solid medium (MRS agar), and a loop was taken from this medium (approximately 1×10^3 CFU mL^{-1}) (CFU = colony-forming unit) and placed in 100 mL of liquid medium (MRS broth) subsequently incubated at 35 °C for 48 h. The microbial concentration was determined over 48 h, at times 0, 4, 8, 12, 16, 24, 28, 36, 40 and 48 h. The quantification of microorganism was performed by surface plating on MRS agar in a Petri dish, using a Spiral Biotech Autoplate® 4000 in the dilution mode (Norwood, MA).

Table 1
Experimental design for encapsulation of *L. rhamnosus* by spray drying.

Experiment	Wall material	Inlet temperature (°C)	Solids (%)	Key of the treatment
1	NRS	135	10	RS-135-10
2			20	RS-135-20
3		145	15	RS-145-15
4		155	10	RS-155-10
5			20	RS-155-20
6	IN	135	10	IN-135-10
7			20	IN-135-20
8		145	15	IN-145-15
9		155	10	IN-155-10
10			20	IN-155-20

NRS = native rice starch; IN = inulin.

2.6. Spray drying process

The blends of wall material and *L. rhamnosus* were spray dried in a laboratory scale dryer (Mod. B-290, Büchi, Switzerland). The airflow used was equivalent to 450–565 L/h. The blends were fed into the drier at a rate of 14 g/min and inlet temperatures of 135, 145 or 155 °C. Prior to feeding the blends into the dryer, a conditioning step with sterile distilled water was performed to maintain stability in the input and output temperatures. Dried powders were collected and immediately placed in sterile containers with saturated $MgCl_2$ salt solution at 4 °C (Rodríguez-Huezo et al., 2007).

2.7. Bulk density

Bulk density takes into account all the pores in and out of the individual particles and can be calculated by placing a mass of particles within a container of known volume. Bulk density was calculated by filling a 10 mL test tube with the powder, and weighing the tube before and after adding the powder. The equation used is as follows (Marousis & Saravacos, 1998):

$$\rho_b = \frac{m}{V}$$

where ρ_b is the bulk density (g/mL), m is the mass of the powder (g), and V is the volume occupied in the cylinder (mL).

2.8. Particle size

The size of encapsulates was determined by laser diffraction analysis using a Mastersize 2000 (Malvern Instruments Ltd., Malvern, Worcestershire, UK). Samples were analyzed using the Hydro 2000s module. Powders were diluted in water to get a final concentration of 0.001% (to achieve 14–15% saturation). Particle size is expressed as the mean diameter ($D[v, 0.5]$) and volume distribution.

2.9. Determination of moisture content and water activity

The moisture content of the samples was measured by hot air oven at 130 °C for 1 h, method 44-16 (AACC, 2001). The water activity of the samples was measured using an AquaLab Mod. 3TE (Pullman, WA) water activity meter.

2.10. Confocal laser scanning microscopy

The study was conducted using a confocal laser scanning microscope (Carl Zeiss, model FluoView™ FV1000, OLYMPUS PME, Miami, FL). The powder was placed on a glass slide, covered with a few drops of solution of rhodamine (chromophore) at a concentration of 0.005% to cause the microorganisms to fluoresce, and placed in darkness for 25 min. After, a coverslip was placed and fixed. Both wall materials were also observed, but no rhodamine was added to discard a possible autofluorescence of the polysaccharides.

2.11. Stability of microencapsulated probiotic at different storage temperatures

The powders were stored in glass bottles at two different temperatures: room temperature (25 °C) and refrigeration (4 °C). For evaluation of the survival of the microorganisms during storage, 1 g of microencapsulated powder was dissolved in 99 mL of peptone, and this blend was spread plated in MRS agar, using a Spiral Biotech Autoplate® 4000 (Norwood, MA). The number of viable bacteria was expressed in CFU/g of microcapsules. The logarithmic value of residual *L. rhamnosus* B442 counts after a storage period, N (log10 CFU/g), was evaluated every 8 days in the powders at different storage conditions and viability was calculated from the microbial reduction using the following equation:

$$\text{Microbial reduction} = \text{Log}\, N_0 - \text{Log}\, N$$

where N_0 is the microbial population after drying and N is the microbial population at different days of storage.

2.12. Morphology

Scanning electron microscopy (SEM) was used to determine the morphology of the encapsulates. Microencapsulated powders were sprinkled on a conductive strip of copper and carbon double adhesion, which was previously set in an aluminum bracket JEOL electron microscope (model JSEM 35CX, Japan Electronic Optical Limited, Tachikawa, Tokio, Japan), then covered with a gold film using an ionizer (DMS-5 Cold Sputterin Module), once covered the samples were placed in a tray and observed.

3. Results and discussion

3.1. Physicochemical characteristics of slurry

Dispersions of native rice starch (NRS) and inulin (IN) at different concentrations were used as wall materials, and density and viscosity were evaluated (Table 2). Both materials showed similar density values at the different concentrations, ranging between 1.03 and 1.08 g/cm^3. A slight increase in density value was observed with increasing concentration of wall material. The density value for both materials is close to that of pure water and therefore this parameter has little influence when the dispersion of the wall material is fed into the spray dryer. The viscosity values were markedly different for both wall materials, showing higher viscosity value for NRS slurries (between 13.2 and 13.6 cP) than for IN solutions (between 2.2 and 2.5 cP). This effect is due to the fact that IN is water soluble while NRS is insoluble in cold water and slurries with higher viscosity values are formed. The solubilization of both wall materials is related with the lower molar mass of IN as compared to NRS. Previous reports indicate that the use of Capsul® (modified starch) at 20% to encapsulate *Beijerinckia* sp. had a viscosity value of 13.6 cP, whereas maltodextrin solution and glucose syrup had viscosity values of 4.0 and 2.5 cP, respectively, showing an influence in the viscosity due to the molar mass of these carbohydrates (Hernández-Carranza & Jiménez-Munguía, 2010). Density and viscosity are important parameters to determine the flow rate of the feed into the spray dryer, and consequently for the physicochemical and functional characteristics of the encapsulates. The viscosity of the solution or slurry of the material affects the viability of the encapsulated microorganism; at low viscosity the flow rate of the feed increases, the drop volume decreases and the contact surface between droplets and hot air in the spray dryer increases, allowing for a rapid drying and reduced thermal treatment on the

Table 2
Physicochemical properties of dispersions of the wall materials.

Wall material	Solids (%)	Density* (g/cm^3)	Viscosity* (cP)
NRS	10	1.03 ± 0.002^f	13.2 ± 0.02^c
	15	1.05 ± 0.002^d	13.3 ± 0.06^b
	20	1.07 ± 0.001^b	13.6 ± 0.14^a
IN	10	1.04 ± 0.001^e	2.2 ± 0.04^f
	15	1.06 ± 0.001^c	2.4 ± 0.13^e
	20	1.08 ± 0.001^a	2.5 ± 0.08^d

* Values are mean ± SEM, $n = 3$; different letters in the same column indicates significant difference ($p < 0.05$). NRS = native rice starch; IN = inulin; 10, 15, 20 = % solids (w/w).

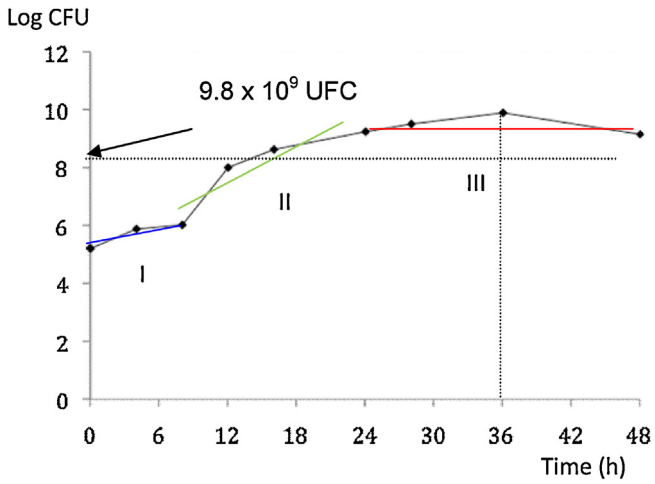

Fig. 1. Growth kinetics of *L. rhamnosus*. CFU = colony-forming unit.

microorganism, thus maintaining their viability (Hernández-Carranza & Jiménez-Munguía, 2010).

3.2. Growth kinetics of L. rhamnosus

Growth kinetics of *L. rhamnosus* was used to determine the time where the maximum number of microorganisms was obtained. Fig. 1 shows the growth curve for *L. rhamnosus*. Under these conditions the maximum cell concentration was reached at 36 h with 9.8×10^9 CFU/g (CFU = colony-forming unit). The microorganism was grown in several flasks and at 36 h, was collected and blended with wall material dispersions to ensure a high number of microorganisms available for encapsulation.

3.3. Encapsulation yield and outlet temperature in the spray dryer

The yield of encapsulated powders ((g wet solids after drying/g wet solids in the dispersion) × 100) after the drying process was higher for NRS than IN, ranging between 65% and 74% for NRS, and between 43% and 54% for IN. This pattern is related with the amount of water present in each wall material as well as the formation of agglomerates in NRS as observed by microscopy. Internal proteins act as bonding agents to produce spherical aggregates resembling popcorn balls. These spherical aggregates are produced when starch granules of small size are spray dried with low amounts of bonding agents such as proteins (Zhao & Whistler, 1994). Water-soluble polysaccharides, such as IN, can be used as wall materials, but lower yield of powder after spray drying is obtained. The advantage of using IN as wall material is that it is not hydrolyzed by digestive enzymes and can serve as substrate to colonic microbiota, thus both, prebiotic and probiotic effects may be attained with IN capsules.

Outlet temperature is an important parameter in the spray drying process, including the encapsulation of microorganisms since the degree to which properties of encapsulated materials are retained depend on the contact time and temperature gradient between the drying air and the material to be dried. NRS slurries and solutions of IN showed an increase in outlet temperature when the solid concentration and inlet temperature increased (Table 3). In general, solutions of IN exhibited higher outlet temperature than NRS slurries at equal solids concentration, except for those blends dried at the highest inlet temperature. The survival of encapsulated microorganism after spray drying is related to the moisture content of the powders, with lower outlet temperature resulting in higher moisture content and increase in the viability

Table 3
Dryer outlet temperatures of the different treatments.*

Treatments**	Outlet temperature (°C)
NRS-10-135	58 ± 0.7^{g}
NRS-20-135	61 ± 1.8^{f}
NRS-15-145	65 ± 1.0^{d}
NRS-10-155	70 ± 1.4^{bc}
NRS-20-155	76 ± 1.6^{a}
IN-10-135	60 ± 0.9^{f}
IN-20-135	63 ± 1.2^{e}
IN-15-145	68 ± 0.9^{c}
IN-10-155	71 ± 0.5^{b}
IN-20-155	77 ± 1.4^{a}

* Values are mean ± SEM, $n = 3$; different letters in the same column indicates significant difference ($p < 0.05$).
** For identification of the treatment see Table 2.

of the microorganisms (Gardiner et al., 2000). However, the moisture content of a powder is an important parameter that influences stability during storage, and operating parameters during drying (inlet temperature and solid concentration) should be optimized to obtain a powder with specific moisture content and consequently an extended shelf life.

3.4. Physicochemical characterization of the encapsulated

The particle size of probiotic powders encapsulated with NRS was similar for all tested conditions, between 5.6 and 5.9 μm (Fig. 2a); solids concentration and inlet temperature did not influence this parameter. The formation of agglomerates of rice starch during spray drying was not affected by operating conditions tested in this work. However, it has been reported that when a bonding

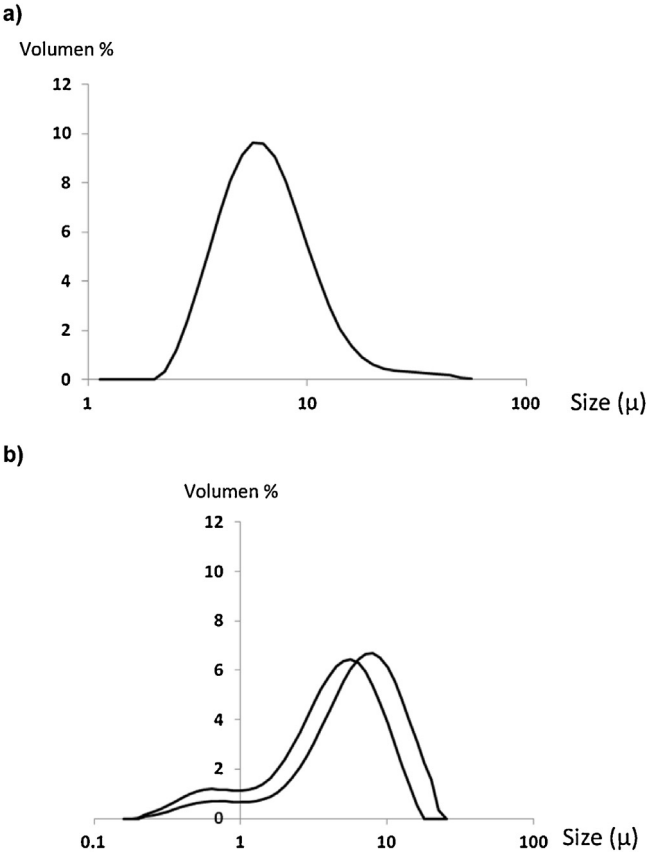

Fig. 2. Particle size distribution of microcapsules: (a) native rice starch and (b) inulin.

Table 4
Physicochemical properties of encapsulated *L. rhamnosus*.*

Treatment**	W_a	Moisture (%)	Density (g/cm^3)
NRS-10-135	0.15 ± 0.01^{abc}	2.7 ± 0.06^{cd}	0.43 ± 0.00^{c}
NRS-20-135	0.13 ± 0.01^{bc}	2.2 ± 0.20^{de}	0.43 ± 0.00^{c}
NRS-15-145	0.16 ± 0.03^{abc}	1.8 ± 0.05^{e}	0.45 ± 0.00^{bc}
NRS-10-155	0.22 ± 0.03^{ab}	3.6 ± 0.07^{a}	0.44 ± 0.00^{c}
NRS-20-155	0.12 ± 0.02^{c}	2.6 ± 0.07^{cd}	0.47 ± 0.00^{b}
IN-10-135	0.24 ± 0.01^{a}	3.9 ± 0.11^{a}	0.55 ± 0.01^{a}
IN-20-135	0.22 ± 0.00^{abc}	3.9 ± 0.07^{a}	0.43 ± 0.00^{cd}
IN-15-145	0.21 ± 0.00^{abc}	2.6 ± 0.04^{cd}	0.44 ± 0.00^{c}
IN-10-155	0.24 ± 0.02^{a}	3.2 ± 0.07^{b}	0.46 ± 0.01^{bc}
IN-20-155	0.20 ± 0.02^{abc}	2.8 ± 0.04^{bc}	0.47 ± 0.01^{b}

* Values are mean ± SEM, $n = 3$; different letters in the same column indicates significant difference ($p < 0.05$).
** For identification of the treatment see Table 2. W_a = water activity.

agent is blended with rice starch the particle size of the encapsulate is affected; for example, a blend of rice starch/Arabic gum produced particle size between 16 and 20 μm and a blend of rice starch/gelatine had particle sizes between 2 and 60 μm (Santos, Fávaro-Trindade, & Grosso, 2005). Another work reported that particle size of encapsulates of rice starch/gelatine around 5 μm, and noted that the concentration and type of bonding agent influenced particle size of the encapsulates (Beirão-da-Costa, Duarte, Moldão-Martins, & Beirão-da-Costa, 2011). Particle size is an important factor during the reactivation of the encapsulated microorganisms since the larger surface area associated with smaller particle sizes allows for greater contact surface between the microorganism and the nutrients essential for their growth (Doherty et al., 2011). The particle size of probiotic powders encapsulated in IN was smaller than NRS, but in the former the processing conditions used during spray drying influenced this parameter (Fig. 2b).

The moisture content of encapsulates with NRS was lower than those with IN (Table 4). This pattern can be due to that IN have shorter chains than NRS, with greater number of OH groups which are able to bind a larger number of water molecules that are not removed during the drying process. A slight effect in moisture content by the temperature in the spray dryer and the solids concentration was observed for both wall materials. The low moisture content of encapsulates suggest stability during storage. Encapsulation of microorganism using spray drying and different wall materials (low-fat milk, blends of milk with polydextrose or IN) presented similar moisture content (Ananta, Volkert, & Knorr, 2004; Corcoran, Ross, Fitzgerald, & Stanton, 2004; Gardiner et al., 2000). Similar pattern was observed for water activity values which ranged between 0.12 and 0.16 for NRS, and between 0.20 and 0.24 for IN. The water activity values of encapsulates for both wall materials are low, corroborating stability during storage. It has been suggested that encapsulates must show water activity between 0.15 and 0.3 and moisture content lower than 5.0% to be microbiologically stable (no growth of external microorganisms), and to prevent caking or recrystallization processes (Corcoran et al., 2004). Higher water activity values (between 0.23 and 0.43) than those determined in our study were determined in encapsulates of *Beijerinckia* sp. using maltodextrin, glucose syrup and resistant starch as wall materials (Nazzaro et al., 2012). The hydrophilic characteristics of the wall material influences the final moisture content and water activity of encapsulates.

The bulk density of encapsulates is low (Table 4) and no effect was observed by the temperature of the dryer, solids concentration and type of wall material. The low density values indicate high porosity of the powders, a characteristic that is important during the application and consumption of the probiotic due to that could be easily solubilized. Additionally, the density of encapsulates is related with the particle size, low-density value indicates small particle size (Fuchs et al., 2006).

3.5. Morphological characteristics

The raw materials used showed different morphological characteristics (Fig. 3a and b) that are important during the encapsulation process. NRS showed the small granules (average 5 μm) and polygonal shape, as reported for various rice cultivars (Wang, Xie, Shi & Xue, 2010). During spray drying, native rice starch produced aggregates, a characteristic noticed for small starch granules when spray

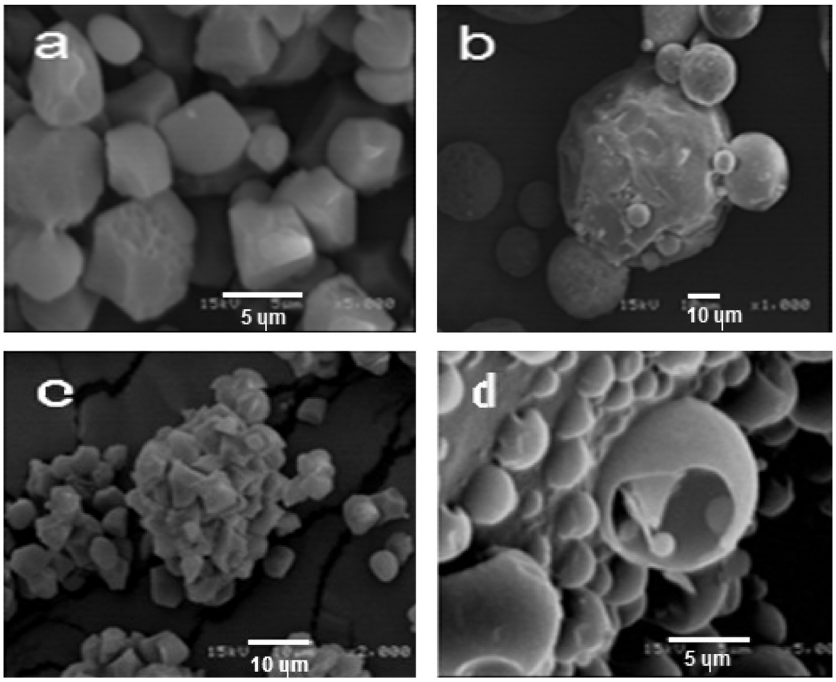

Fig. 3. Scanning electron micrograph of: (a) native rice starch, (b) inulin from chicory, (c) agglomerates formed microcapsules with native rice starch (20%, w/w) and the dryer inlet temperature of 135 °C, and (d) microcapsules formed with chicory inulin to 20% (w/w) and the dryer inlet temperature of 155 °C.

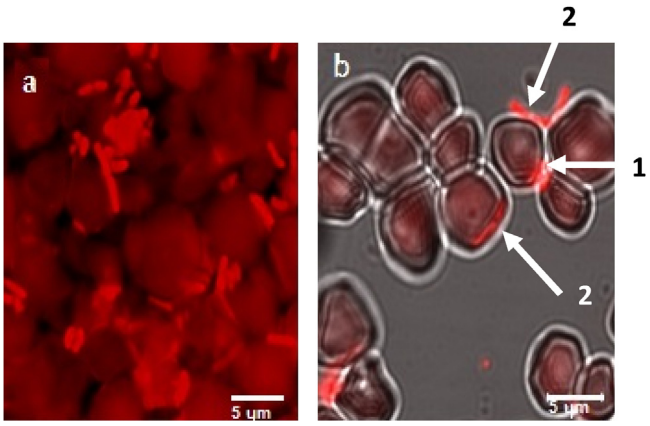

Fig. 4. Laser scanning confocal micrograph of: (a) *L. rhamnosus* in a cluster consisting of native rice starch and (b) encapsulated inulin.

dried in the presence of bonding agents; however, some studies have reported that the protein present in native starch granules may promote the formation of aggregates (Beirão-da-Costa et al., 2011; González-Soto, de la Vega, García-Suarez, Agama-Acevedo, & Bello-Pérez, 2011). These aggregates (Fig. 3c) carry the microorganisms that can in turn be released from the porous structure of the starch granules. This was corroborated by staining the microorganisms in the aggregates (Fig. 4a). Microorganisms were observed within the interstitial spaces and at the periphery of the aggregates. The morphology of the IN showed spherical shapes with diverse sizes and irregular surface (Fig. 3b). The shape and size on IN encapsulates did not change, but changes in the surface (smooth surface), and the presence of hollow "eggshell"-like structures, were observed (Fig. 3d). Morphological characteristics similar to those reported for encapsulates with different carbohydrates such as fructans, glucose syrup and maltodextrins were obtained (Hernández-Carranza & Jiménez-Munguía, 2010; Rodríguez-Huezo et al., 2007). Confocal microscopy images of IN encapsulates show presence of the microorganisms within the interstitial spaces of the inulin spheres (see arrow 1 in Fig. 4b) and at the periphery of those spheres (see arrow 2 in Fig. 4b). The staining test corroborated that IN encapsulated the microorganisms in a different manner than NRS, which could in turn provide different release properties and functionality between NRS and IN.

3.6. Viability of probiotic microorganisms in the encapsulates

Overall, the lowest reduction in viability of encapsulated microorganisms was obtained with NRS (Table 5). The formation of agglomerates with native rice starch, as was observed in the microscopy study, better protected the microorganisms. The inlet temperature of the spray dryer affected the viability with both wall materials, with higher temperature causing greater reduction in viability due to thermo-sensitivity of the microorganisms. The concentration of the slurry affected the viability due to that higher concentration provoked a minor reduction in the viability. The amount of solids in the slurry that is introduced in the spray dryer limited the heat transfer and consequently increased the viability. Encapsulation of microorganisms using maltodextrins with an inlet temperature of 135 °C produced logarithmic reduction of viable microorganisms between 0.16 and 0.23 (Hernández-Carranza & Jiménez-Munguía, 2010), which was lower to those determined in our study with NRS and IN. Reduction in the number of the microorganisms lower than 2 logarithmic cycles for populations larger than

Table 5
Viability of *L. rhamnosus* at the end of spray drying process using rice starch and inulin at different concentrations.*

Treatment**	No. of viable cells		Log reduction population ($\text{Log } N_0/N$)*
	Initial population N_0 (CFU/g) (E+08)	Population after drying N (CFU/g) (E+08)	
NRS-10-135	18.2	9.69	0.27 ± 0.07^{c}
NRS-20-135	23.5	9.04	0.42 ± 0.05^{bc}
NRS-15-145	23.9	12.00	0.30 ± 0.03^{c}
NRS-10-155	15.8	6.65	0.38 ± 0.04^{bc}
NRS-20-155	16.2	8.06	0.31 ± 0.06^{bc}
IN-10-135	15.0	4.56	0.52 ± 0.04^{b}
IN-20-135	17.2	8.26	0.32 ± 0.03^{bc}
IN-15-145	15.2	8.14	0.27 ± 0.03^{c}
IN-10-155	24.4	4.64	0.72 ± 0.11^{a}
IN-20-155	22.1	8.54	0.41 ± 0.05^{bc}

* Values are mean ± SEM, $n = 6$; different letters in the same column indicates statistically significant difference ($p < 0.05$).
** For identification of the treatment see Table 2. CFU = colony-forming unit.

Table 6
Viability of encapsulated *L. rhamnosus* stored under refrigeration (4 °C) and room temperature (25 °C) for 32 days.*

Treatment**	Microbial population after drying process $\text{Log } N_0$ (CFU/g)	Microbial population at day 32 $\text{Log } N$ (CFU/g)		Microbial reduction at day 32* ($\text{Log } N_0 - \text{Log } N$)	
		4 °C	25 °C	4 °C	25 °C
NRS-10-135	8.99	8.58	8.36	0.40 ± 0.09^{de}	0.62 ± 0.09^{d}
NRS-20-135	8.96	8.77	8.79	0.19 ± 0.06^{fg}	0.17 ± 0.06^{ef}
NRS-15-145	9.08	8.28	8.74	0.80 ± 0.07^{cd}	0.34 ± 0.11^{de}
NRS-10-155	8.82	8.50	8.29	0.33 ± 0.13^{ef}	0.54 ± 0.14^{d}
NRS-20-155	8.91	8.76	8.52	0.15 ± 0.05^{fg}	0.38 ± 0.09^{de}
IN-10-135	8.66	7.80	6.92	0.86 ± 0.02^{c}	1.74 ± 0.05^{b}
IN-20-135	8.92	8.29	6.69	0.63 ± 0.15^{cde}	2.23 ± 0.12^{a}
IN-15-145	8.91	7.08	6.65	1.83 ± 0.06^{a}	2.26 ± 0.18^{a}
IN-10-155	8.67	7.84	6.78	0.83 ± 0.03^{c}	1.89 ± 0.05^{b}
IN-20-155	8.93	7.62	7.70	1.32 ± 0.13^{b}	1.23 ± 0.08^{c}

* Values are mean ± SEM, $n = 6$; different letters in the same column indicates statistically significant difference ($p < 0.05$).
** For identification of the treatment see Table 2. CFU = colony-forming unit.

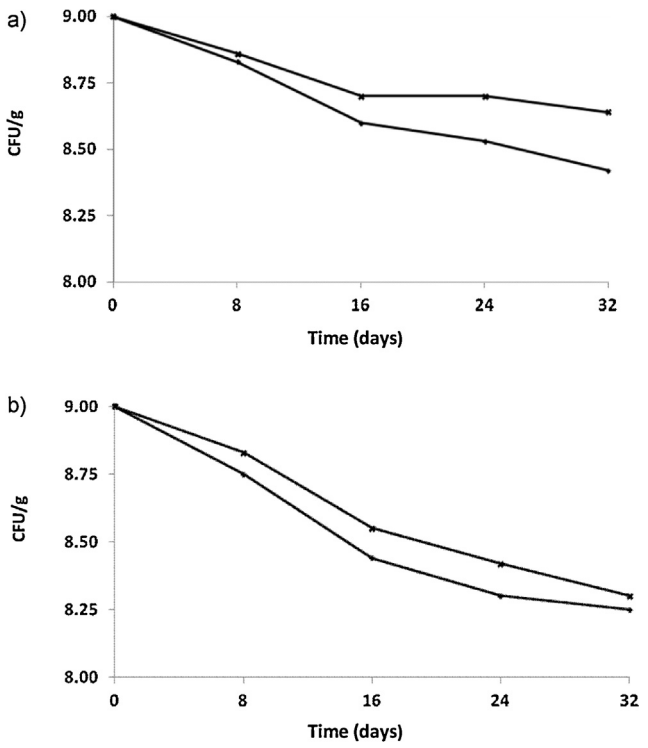

Fig. 5. Viability of *L. rhamnosus* encapsulated: (a) native rice starch (NRS-20-135) and (b) inulin (IN-20-155), stability under storage conditions 4 y 25 °C. CFU = colony-forming unit.

1×10^7 CFU/g in the microcapsules is adequate for incorporation into a food (De Vos, Faas, Spasojevic, & Sikkema, 2010; Rokka & Rantamäki, 2010). NRS as wall material adequately protects the microorganisms during spray drying process and results in microcapsules with high viability.

3.7. Stability of encapsulates during storage

The reduction in the number of microorganisms after 32 days of storage at two different temperatures was higher for IN encapsulates than for NRS (Table 6). This observation suggests that the formation of agglomerates with NRS provides better protection to microorganisms, and possibly by the formation of a thin film of amylose and some external long chains of amylopectin apart out from starch granules during the spray drying. In general, higher reduction was observed in powders of both colloids stored at 25 °C than 4 °C due to the metabolic activity of the microorganism being higher at 25 °C, thus requiring substrates to maintain the viability (Fig. 5). Powders of NRS at the same concentration but at different drying temperature stored at both temperatures had similar reduction value in the number of microorganisms, and those dried at the lowest temperature showed an effect by the concentration of the colloids. Higher protection was observed at higher concentration due to that the reduction in the number of microorganisms was lower. At the highest drying temperature (155 °C) heat transfer is not limited by solids concentration causing similar damage to the microorganisms.

4. Conclusions

The use of both prebiotics colloids (NRS and IN) without bonding agent is suitable for protecting *L. rhamnosus* during spray drying. The morphological and physicochemical properties conferred by each colloid, along with the varying degree of protection provided by each colloid, suggests that the release of the encapsulated microorganism in the gastrointestinal system or in the food matrix could potentially differ depending on the wall material used in the encapsulation process. The viability and stability results indicated that both colloids successfully protected to *L. rhamnosus* during spray-drying.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgements

The authors thank the support from SIP-IPN, COFAA-IPN and EDI-IPN. One of the authors (SVAR) also acknowledges the scholarship from CONACYT-Mexico.

References

AACC. (2001). *American Association of Cereal Chemists: Approved methods of the AACC* (10th ed.). St. Paul, MN: AACC.
AOAC. (1995). *Association of Official Analytical Chemists: Official methods of analysis* (10th ed.). Washington, DC: AOAC.
Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology*, *18*(5), 240–251.
Ananta, E., Volkert, M., & Knorr, D. (2004). Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. *International Dairy Journal*, *15*, 399–409.
Beirão-da-Costa, S., Duarte, C., Moldão-Martins, M., & Beirão-da-Costa, M. L. (2011). Physical characterization of rice starch spherical aggregates produced by spray-drying. *Journal of Food Engineering*, *104*, 36–42.
Boza, Y., Barbin, D., & Scamparini, A. R. P. (2004). Survival of *Beijerinckia* sp. microencapsulated in carbohydrates by spray-drying. *Journal of Microencapsulation*, *21*, 15–24.
Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Review: Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering*, *2011*(104), 467–483.
Chavez, B. E., & Ledeboer, A. M. (2007). Drying of probiotics: Optimization of formulation and process to enhance storage survival. *Drying Technology*, *25*, 1193–1201.
Crittenden, R., Laitila, A., Forssell, P., Matto, J., Saarela, M., Mattila-Sandholm, T., et al. (2001). Adhesion of bifidobacteria to granular starch and its implications in probiotic technologies. *Applied and Environmental Microbiology*, *67*, 3469–3475.
Corcoran, B. M., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2004). Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. *Journal of Applied Microbiology*, *96*, 1024–1039.
Da Rosa Zavareze, E., Mello El Halal, S. L., de los Santos, D. G., Helbig, E., Mascarenhas Pereira, J., & Guerra Dias, A. R. (2012). Resistant starch and thermal, morphological and textural properties of heat–moisture treated rice starches with high-, medium- and low-amylose content. *Starch/Stärke*, *64*, 45–54.
De Vos, P., Faas, M. M., Spasojevic, M., & Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal*, *20*, 292–302.
Desmond, C., Ross, R. P., 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.
Doherty, S. B., Gee, V. L., Ross, R. P., Stanton, C., Fitzgerald, G. F., & Brodkorb, A. (2011). Development and characterization of whey protein micro-beads as potential matrices for probiotic protection. *Food Hydrocolloids*, *25*, 1604–1617.
Favaro-Trindade, C. S., & Grosso, C. R. F. (2002). Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile. *Journal of Microencapsulation*, *19*, 485–494.
Figueroa-Gonzalez, I., Quijano, G., Ramırez, G., & Cruz-Guerrero, A. (2011). Probiotics and prebiotics – Perspectives and challenges. *Journal of the Science of Food and Agriculture*, *91*, 1341–1348.
Fuchs, M., Turchiuli, C., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Maillard, M. N., et al. (2006). Encapsulation of oil in powder using spray drying and fluidized bed agglomeration. *Journal of Food Engineering*, *75*, 27–35.
Fuentes-Zaragoza, E., Sánchez-Zapata, E., Sendra, E., Sayas, E., Navarro, C., Fernández-López, J., et al. (2011). Resistant starch as prebiotic: A review. *Starch/Stärke*, *63*, 406–415.
Gardiner, G. E., O'ullivan, E., Kelly, J., Auty, M. A., Fitzgerald, G. F., Collins, J. K., et al. (2000). Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. *Applied and Environmental Microbiology*, *66*, 2605–2612.
Geankoplis, C. J. (1998). *Transport processes and unit operations* (3rd ed.). Singapore: Prentice Hall International.
González-Soto, R. A., de la Vega, B., García-Suarez, F. J., Agama-Acevedo, E., & Bello-Pérez, L. A. (2011). Preparation of spherical aggregates of taro starch granules. *LWT – Food Science and Technology*, *44*, 2064–2069.

Goderska, K., & Czarnecki, Z. (2008). Influence of microencapsulation and spray drying on the viability of *Lactobacillus* and *Bifidobacterium* strains. *Polish Journal of Microbiology*, *7*, 135–140.

Hernández-Carranza, P., & Jiménez-Munguía, M. T. (2010). Propiedades funcionales y aplicaciones industriales de los fructo-oligosacáridos. *Temas Selectos de Ingeniería de Alimentos*, *4*(1), 1–8.

Ivanovska, P. T., Petruševska-Tozi, L., Dabevska Kostoska, M., Geškovski, N., Grozdanov, A., Stain, C., et al. (2012). Microencapsulation of *Lactobacillus casei* in chitosan-Ca-alginate microparticles using spray-drying method. *Macedonian Journal of Chemistry and Chemical Engineering*, *31*, 115–123.

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

Kalyani Nair, K., Kharb, S., & Thompkinson, D. K. (2010). Inulin dietary fiber with functional and health attributes – A review. *Food Research International*, *26*, 189–203.

Marousis, S. N., & Saravacos, G. D. (1998). Density and porosity in drying starch materials. *Journal of Food Science*, *55*, 1367–1372.

Nazzaro, F., Orlando, P., Fratianni, F., & Coppola, R. (2012). Microencapsulation in food science and biotechnology. *Current Opinion in Biotechnology*, *23*, 182–186.

Nedovica, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, *1*, 1806–1815.

O'Riordan, K., Andrews, D., Buckle, K., & Conway, P. (2001). Evaluation of microencapsulation of a *Bifidobacterium* strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology*, *91*, 1059–1066.

Palma-Rodriguez, H. M., Agama-Acevedo, E., Mendez-Montealvo, G., Gonzalez-Soto, R. A., Vernon-Carter, E. J., & Bello-Pérez, L. A. (2012). Effect of acid treatment on the physicochemical and structural characteristics of starches from different botanical sources. *Starch/Stärke*, *64*, 115–125.

Picot, A., & Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal*, *14*, 505–515.

Puncha-Arnon, S., & Uttapap, D. (2013). Rice starch vs. rice flour: Differences in their properties when modified by heat–moisture treatment. *Carbohydrate Polymers*, *91*, 85–91.

Rodríguez-Huezo, M. E., Durán-Lugo, R., Prado-Barragán, L., Cruz-Sosa, F., Lobato-Calleros, C., Alvarez-Ramírez, J., et al. (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.

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: Challenges for industrial applications. *European Food Research and Technology*, *231*, 1–12.

Santos, A. B., Fávaro-Trindade, C. S., & Grosso, C. R. (2005). Preparo e atomização de microcápsulas de oleoresina de páprica obtidas por atomização. *Ciência e Tecnolgia de Alimentos*, *25*, 322–326.

Zhang, G., Venkatachalam, M., & Hamaker, B. (2006). Structural basis for the slow digestion property of native cereal starches. *Biomacromolecules*, *7*, 3259–3266.

Zhao, J., & Whistler, R. L. (1994). Spherical aggregates of starch granules as flavor carriers. *Food Technology*, *48*, 104–105.

Wang, L., Xie, B., Shi, J., & Xue, S. (2010). Physicochemical properties and structure of starches from Chinese rice cultivars. *Food Hydrocolloids*, *24*, 208–216.