THERMAL STABILIZATION OF PROBIOTICS BY ADSORPTION ONTO POROUS STARCHES

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Abstract

Industrial processing factors, such as temperature, compromise the viability of probiotic cells. Objective was to develop a system to thermally stabilize probiotic bacteria based on porous starches and using biopolymers as coating materials (gelatinized starch, guar gum and xanthan gum). Porous starches from corn and rice starches, having controlled number and size of porous were used as supporting material. Scanning electron microscopy confirmed the adsorption of the microorganism, leading microcapsules with corn starch but aggregates with rice starch. Surface pores of rice starch increased the encapsulation yield of rice starch around 10%, but that effect was not observed in porous corn starch. The highest encapsulation yield was obtained with porous starches coated with gelatinized starch, which ranged from 92-100%. Microencapsulates made with porous starches with small pores, like the ones obtained with α -amylase, and coated with gelatinized starch resulted in the highest thermal resistance at 55 °C.

Keywords: Porous starch; enzymes; probiotics; amylase; amyloglucosidase; *L. plantarum*; thermal stability.

1. Introduction

The emphasis on the use of food to promote well-being and a healthy state have driven to the development of the so-called "functional foods" (Roberfroid, 2000). Nearly all segments of the food industry offer functional products, opening the door to dairy products, soft drinks, juices, pastries and infant food (Miñana & Serra, 2009), with functional ingredients such as probiotics, prebiotics, vitamins and minerals (Stanton et al., 2001). Nevertheless, a constant major challenge is to ensure that these products preserve the viability of probiotic microorganisms under the harsh conditions associated to product processing. In this regard, microencapsulation techniques are used to protect microorganisms in food.

In general, starches, particularly modified starches, have been used as coating materials for encapsulation. A non-chemical way to modify starch granules is by applying enzymatic treatment using amylolytic enzymes. Those modified starches led to porous molecules with great adsorbent capacity, due to their large surface area (Zhang et al., 2012), but also those pores provide an expandable space that could be used as a protective microenvironment for probiotic encapsulation. It might be expected that the probiotic bacteria would be physically adsorbed in the pores and cavities without any covalent binding, allowing their complete released in a sustained manner (Li, Thuy Ho, Turner, & Dhital, 2016) whenever having the right size and shape of pores. However, size and shape of the microporous structure largely depends on the amylase type and amylolysis level. Dura, Błaszczak, and Rosell (2014) studied the enzymatic modification of corn starch by using α -amylase (AM) and amyloglucosidase (AMG) enzymes revealing the formation of superficial micropores with diverse pore size depending on the amylolytic enzyme. Authors concluded that AMG yielded starch granules with more abundant and larger pores than those obtained after AM treatment. Recently, Benavent-Gil and Rosell (2017a) compared the effect of a range of amylases on the properties of corn starch, also taking into account the impact of the enzymatic level, revealing that the number and size of the pores could be modulated by controlling the amylase type and level. Additionally, the intrinsic structural characteristics of starches from different botanical origin can offer extended possibilities for obtaining porous starches. In fact, studies carried out with different amylolytic enzymes on cereal and tuber starches indicated that starches of cereal origin have deeper and larger pores compared to the superficial cavities observed in tuber starches after enzymatic modification (Benavent-Gil & Rosell, 2017b). Therefore, the resulting porous starches

might have different technological performance and, consequently, different industrial applications.

Further stability of the probiotic cells can be obtained by applying coating materials. This technology allows enclosing the probiotics cells inside the microcapsules that are subsequently coated by an additional layer. Overall, hydrolyzed starches (Brinques & Ayub, 2011; Lahtinen, Ouwehand, Salminen, Forssell, & Myllärinen, 2007; Li et al., 2016; Xing et al., 2014)), porous starches and starches/alginate (Brinques & Ayub, 2011; Lahtinen et al., 2007; Li et al., 2016; Xing et al., 2014) have been the elected encapsulating agents for many authors. Meanwhile, hydrocolloids are frequently used for coating microcapsules. Hydrocolloids produce a gel network structure that can easily adhere to the surface of the microcapsules to control external and internal mass transfer (Morreale, Garzón, & Rosell, 2017). This additional shell to the encapsulated cells can provide better barrier and protection against harsh environmental conditions (Li et al., 2016; Xing et al., 2015). Nevertheless, a careful selection of the coating materials must be made to obtain capsules with different physical properties. For instance, the type and level of the hydrocolloid applied for coating can alter the starch granule properties (Gularte & Rosell, 2011).

In spite of the great possibilities that porous starches could offer for probiotics production, as far as authors knowledge, up to now there are no previous studies about the effect of different coating materials on the stability of microcapsules based on enzymatically modified starches with different structure. Therefore, the objective of this study was to identify the potential of controlled pore size starches from different botanical sources, obtained in a previous study (Benavent-Gil & Rosell, 2017b), as carriers of probiotics. Particularly, to investigate the role of different enzymatic treatments (AMG and AM) on two different starches (corn and rice) on the *Lactobacillus plantarum* viability, as probiotic microorganism, during the encapsulation process and to establish the possible correlation between the morphological properties and the thermal stability of the bacteria within the microcapsules. The influence of different coating materials on the survival rate of *L. plantarum* was also evaluated during exposure to heating treatment at different times.

2. Materials and methods

2.1. Starch samples

The starch samples were selected from previous studies (Benavent-Gil & Rosell, 2017a, 2017b). The starch sources were corn starch (C) (Miwon, Seoul, Korea) and intermediate amylose rice starch (R) (Sigma-Aldrich, Spain). Their respective enzymatic modifications were carried out with amyloglucosidase (AMG) (EC 3.2.1.3) and fungal α -amylase (AM) (EC 3.2.1.1) treatment (Novozymes, Bagsværd, Denmark), using 16.5 U AMG / g starch and 11 U AM / g starch. The selected starches evinced the microstructure characteristics summarized in Table 1, which were obtained from the image analysis of the scanning electron micrographs using ImageJ software (ImageJ, UTHSCSA Image Tool software). Surface starch characteristics were previously reported by (Benavent-Gil & Rosell, 2017a, 2017b). Granule and pore size, as well as pore frequency (ratio of the sum of the areas of all the pores in a granule and the granule area).

2.2. *Strains, media and growth conditions*

The bacterial strain used in this study was *Lactobacillus plantarum* CECT 230. The strain was grown in de Man, Rogosa and Sharpe (MRS) broth (Scharlab, Barcelona, Spain) at 30 °C for 24 h. Cells were harvested and washed by centrifugation at 4,000 x g for 10 min and resuspended with sterile peptone water, resulting in a cell suspension containing approximately 2×10^{10} CFU mL⁻¹.

2.3. *Encapsulation of Lactobacillus plantarum cells*

L. plantarum cells were encapsulated in the native and modified starches. Starch (2 g) was transferred into sterile tubes containing 6 mL of bacterial culture. The encapsulation process was carried out in four different stages: microorganism adsorption (S1), vacuum filtering (S2), freezing (S3) and freeze drying (S4). Process was as follows: the mixture was kept in a shaking water bath (600 x g) at 30 °C for 90 min (S1). Then, samples were vacuum filtered through Whatman n° 2 filter paper mounted in a Buchner filter (S2). After that, microcapsules were frozen and kept at -20 °C for 1 h (S3). Microcapsules were freeze-dried for 24 h and kept at 4 °C for subsequent analyses (S4). The encapsulation process was conducted in duplicate, separately using two batches of prepared starches. When coating was applied onto the surface of the microcapsules the same procedure described above was carried out, but coating material was added to the microcapsules in the stage S3, before freezing. Specifically, microcapsules and coating material were gently homogenized (3 mL g^{-1} coating material) with a Polytron Ultraturrax homogenizer IKA-T18 (IKA works, Wilmington, USA) for 0.5 min at speed 3 and then frozen.

2.4. *Edible coating material preparation*

Three different coating material were prepared. Gelatinized starch (GS) was prepared by heating native starch (6% w/v) in water for 15 min at 90 °C. Guar gum (GG) (Guar gum – 3500 from EPSA, Spain) and xanthan gum food grade (GX) (Jungbunzlauer, Austria) suspensions (2%, w/v) were used as coating material, separately. Preliminary tests were carried out to optimize the level of coating material suspension in order to cover the largest proportion of granule.

2.5. *Encapsulation yield*

To determine the encapsulation yield (EY) at the different process stages (S1-S4) microorganism viability in starch samples was studied by plate counting on MRS agar. The microcapsules (0.10 g) were first added into 0.9 mL peptone water (0.1% w/v) containing pancreatin (0.9 mg/ 100 mg starch). The pancreatin was added to hydrolyze the starch releasing the encapsulated bacteria (Li et al., 2016). The volume of 0.1 mL of decimal serial dilutions in peptone water were plated in duplicate on MRS agar and incubated at 30 °C for 48 h. The microbial count data was expressed as decimal-log of colony-forming units per gram (CFU g⁻¹). Encapsulation yield (EY) (%) was calculated by using the equation of Ashwar, Gani, Gani, Shah, and Masoodi (2018)

$$EY = N/N_0 \times 100$$

Where N is the log cell count (CFU/g) of viable entrapped cells released from the microcapsules, and N₀ is the log cell count (CFU/g) of free cells added to the production of microcapsules.

2.6. *Scanning electron microscopy (SEM)*

A JSM 5200 scanning electron microscope (SEM) (JEOL, Tokyo, Japan) was used to visualize the distribution of probiotic bacteria in native starches and enzymatically modified starches. Samples were coated with gold in a vacuum evaporator (JEE 400, JEOL, Tokyo, Japan) prior to observation. The obtained samples were examined at an accelerating voltage of 10 kV and magnified 3,500x times.

2.7. *Thermal stability studies*

The heat resistance of the encapsulated *L. plantarum* was evaluated by a thermal treatment at 55 °C for 20 and 35 min, which were set up in preliminary studies (data not showed).

Before treatment, 100 mg microcapsules were inoculated into 20 mL peptone water (0.2%, w/v) and introduced in Thermal-Death-Time (TDT) stainless steel tubes. A thermocouple connected to a data logger was introduced through the sealed screwed top to follow the process temperature. After 0, 20 and 35 min incubation, the tubes were rapidly cooled in an ice-water bath to room temperature, centrifuged and supernatant removed. The pellet obtained was used to determine the total number of viable cells as described in Section 2.5.

2.8. Statistical analysis

The data reported are the mean of replicates and expressed as a mean $\pm$ standard deviation. Statistical analyses were carried out with Fisher's least significant differences test with a significance level of 0.05. Pearson correlation coefficient (r) and P -value were used to indicate correlations and their significance using Statgraphics Centurion XV software (Bitstream, Cambridge, N). The correlation coefficient was classified in different levels of correlation: perfect ($|r| = 1.0$), strong ($0.80 \leq |r| \leq 1.0$), moderate ($0.50 \leq |r| \leq 0.80$), weak ($0.10 \leq |r| \leq 0.50$), and very weak (almost none) correlation ($|r| \leq 0.10$).

3. Results

3.1. Microstructure of the microcapsulates

The microstructure of native and porous starches was widely described in previous studies (Benavent-Gil & Rosell, 2017a, 2017b), but since the morphology of microcapsules from raw and modified materials could affect the encapsulation process, some details about those starches are included in Table 1. Figure 1 exhibits the microcapsules obtained after S4 stage. SEM was used to investigate the distribution of *L. plantarum* in modified and unmodified starch materials (Fig. 1). As expected, the microorganism adhesion onto the granular surface was clearly visualized in all samples. Nevertheless, the microcapsules obtained from corn or rice sources revealed a markedly different pattern. In the corn microcapsules (Fig. 1 A, B, C), the microorganism adhesion was more pronounced than onto the rice granular surface (Fig. 1 D, E, F). After freeze drying (S4), microcapsules from treated and untreated rice starches gave aggregates, whereas in the case of corn they appeared as individual entities. Those morphological characteristics have been previously reported for native starches and rice based encapsulates obtained by spray drying process (Avila-Reyes, Garcia-Suarez, Jiménez, San Martín-Gonzalez, & Bello-Perez, 2014; Horstmann, Belz, Heitmann, Zannini, & Arendt, 2016), and those starchy aggregates have

been related to the residual protein nearby rice starch granules (Beirão-da-Costa, Duarte, Moldão-Martins, & Beirão-da-Costa, 2011).

Table 1. Structural characteristics of native and modified starches used as supporting materials. C: Corn, R: rice, AMG: porous starch from corn or rice obtained with amyloglucosidase, AM: porous starch from corn or rice obtained with α -amylase.

	Granule Size (μm^2)	Pore Size (μm^2)	Pore frequency (%)
C	87.68	n.d	n.d
C-AMG	87.68	0.59	4.47
C-AM	87.68	0.13	1.57
R	17.55	n.d	n.d
R-AMG	17.55	0.19	3.69
R-AM	17.55	0.03	0.43

n.d.: Not detected.

The native starches from corn and rice showed lower adhesion onto the granule surface (Fig. 1 A, D). In contrast, higher load of bacteria was observed when using AMG or AM treated starches (Fig. 1 B, C, E, F), which exhibited a porous surface with internal cavities due to the 'inside out' hydrolysis (Dhital, Shrestha, & Gidley, 2010). This is an indication that the enzymatic modification and the consequent formation of deep pores affected the adhesion capacity of the bacteria onto the surface of the granules. It has been described that unmodified starches only were able to adsorb bacteria onto the granular surface (Conrad, Miller, Cielenski, & de Pablo, 2000). Meanwhile, superficial holes might facilitate the bacterial entrapment, due to the expanded space, which can be filled with bacteria (Li et al., 2016). In this sense, Wu, Du, Ge, and Lv (2011) demonstrated an increase in the oil adsorption capacity related to the degree of hydrolysis, i.e., greater degree of hydrolysis produced during the formation of porous starch led to larger surface for the adsorption of different components. In addition, Zhang et al. (2012) stated that when producing porous starches, enzyme concentration should be optimized to increase their adsorption capacity, because excessive hydrolysis would be detrimental.

Fig. 1. SEM micrographs of native corn (A) and rice (D) starches and the resulting microencapsulated *L. plantarum* with different supporting materials: porous corn starch obtained with amyloglucosidase (B); porous corn starch obtained with amylase (C); porous rice starch obtained with amyloglucosidase (E); porous rice starch obtained with amylase (F).

3.2. Effect of enzymatic treatment on encapsulation yield

Encapsulation yields (EY) (%) onto the different starches through the different stages are given in Fig. 2. Porous starches after AMG or AM treatment of corn or rice were used as the supporting material. The statistical analysis indicated that the starch source had a significant ($P < 0.05$) effect on EY, but not the enzymatic treatment applied to obtain the porous starches. Nevertheless, when analyzing each microencapsulation stage, significant differences in the EY were observed due to the starch source at stage S1, meanwhile the enzyme type prompted significant different effect at S1 and S4 stages.

Regarding S1 stage, the encapsulation efficiency of *L. plantarum* was similar for the native starches regardless of their origin, with an EY of 93.02% for native corn starch and 92.92% for native rice starch. Porous rice starch did not improve the encapsulation efficiency compared to its native counterpart, which suggested that the increase in porosity did not improve the adsorption, supporting the importance of the granules aggregates to entrap the microorganism. Conversely, in the case of porous corn starches, the EY of *L. plantarum* reached 100%, indicating better adsorption due to the superficial pores. Nevertheless, no significant difference ($P > 0.05$) in the EY was observed due to the

procedure for obtaining porous with AM or AMG, thus the pore size and pore frequency hardly affected the microorganism adsorption onto the surface. It can be also taken into account that Crittenden et al. (2001) described the adherence of different strains of lactic acid bacteria onto starch, which was promoted by the starchy hydrolysis products released from the action of the strains on the starch. The results obtained in the present study indicate that the cell adhesion capacity for *L. plantarum* depends on the supporting material used for the microencapsulation. Similar results have been previously described for the oil absorption capacity of porous starches from different sources, concluding that the pore size plays a fundamental role for the adsorption of oil and water molecules (Benavent-Gil & Rosell, 2017a, 2017b).

Fig. 2. Effect of supporting materials (C: Corn, R: rice, AMG: porous starch obtained with amyloglucosidase, AM: porous starch obtained with α -amylase) on *L. plantarum* encapsulation yield at different process stages (S1. immediately after starch inoculation; S2. after vacuum filtering; S3. after freezing; S4. after freeze drying). Mean bars with different letters within the same supporting material differed significantly ($P < 0.05$).

During S2 and S3 stages, which comprised the vacuum filtering and freezing, samples showed similar trends. The EY remained constant during the S2 and S3 stages, except for native rice sample that showed an increase in the viability of the microorganisms after filtration, which remained constant during freezing. Similar results were found by Heidebach, Först, and Kulozik (2010).

Regarding S4, freeze drying is frequently used as an effective way to produce probiotic products with high stability rates during preservation and convenient handling (Li et al., 2016). Nevertheless, S4 stage induced a decrease of the encapsulation efficiency, except in the case of porous rice starches. The lowest EY was obtained with native starches, presenting an encapsulation efficiency of 87.67% for corn and 84.91% for rice starch. A possible explanation might be that the absence of pores allowed the inclusion of cells only at the very superficial level of the microcapsule. Bacteria adhered onto the granular surface presumably had greater exposure to low temperature and the subsequent formation of ice crystals (Li et al., 2016). The removal of water during the sublimation process after the formation of intracellular ice crystals during the freezing process can damage the cellular membrane (Conrad et al., 2000). Thus, the absence of pores resulted in the reduction microorganism viability during the freeze drying process. The enzymatic modification of corn starch did not result in an increase of the EY, having similar yields compared to the native starch ($87 \pm 3 \%$). Comparable results were obtained for native and porous corn starches obtained with pancreatic α -amylase, pancreatin, and fungal α -amylase, without significant differences on the relative survival (Li et al., 2016). Nevertheless, the enzymatic treatment of rice starch improved the EY (91.86 ± 0.67 and $91.24 \pm 2.14\%$ for AMG and AM treatments, respectively), observing an increase in the viable cell count compared with the native starch ($84.91 \pm 3.00 \%$). As it was observed in the micrographs, porous and native rice starches formed aggregates that might protect the microorganisms located within the interstitial spaces (Avila-Reyes et al., 2014). Nevertheless, in the present study, the formation of aggregates in the native rice starch was not enough to improve the protection of the microorganisms after freeze drying process. Porous rice starches showed higher EY after freeze drying process than its native counterpart. Thus, it can be assumed that in rice, porous structure correlates with an increase in EY (Li et al., 2016). The results also suggested that porous structure in rice starch together with its ability to agglomerate, contributed to the microorganism protection.

3.3. *Effect of coating material on encapsulation yield*

In order to provide better protection to the microencapsulated cells, different polymers like gelatinized starch, guar gum and xanthan gum were used as coating materials. The protective effect against the damage induced during the freeze drying process is presented in Fig. 3 (SEM micrographs can be displayed in supplementary material). After freeze drying stage, the *L. plantarum* EY when coated with gelatinized starch, GG or GX were

92-100%, 75.76-98.79% and 84.09-99.18%, respectively, versus 84.91-91.86% found in the absence of coating materials. Generally, drying processes have a typical survivability rate around 70-85% (Lahtinen, Ouwehand, Salminen, & von Wright, 2011). The higher survival rate of the cells confirmed that coating materials maintained the integrity of cells and enhanced the microencapsulated cell number.

Fig. 3. Effect of coating material on *L. plantarum* encapsulation yield (EY) at S4 (freeze drying stage). Different letters inside the symbols differ significantly ($P < 0.05$). C: Corn, R: rice, AMG: porous starch obtained with amyloglucosidase, AM: porous starch obtained with α -amylase.

Coating polymers had a significant effect on *L. plantarum* EY ($P < 0.01$). Gelatinized starch was the unique coating material that increased the EY, regardless starch source and enzymatic treatment compared to non-coated encapsulated samples (Fig. 3). This result is in agreement with findings of Xing et al. (2015), who indicated that pores in the modified starch and the presence of mannitol, glycerol and sodium alginate as complex coating materials offer better protection for *L. acidophilus* cells. Nevertheless, results indicated that protective effect was greatly dependent on the coating material. In fact, GX was only effective with the microcapsules from porous starches, with the exception of those from

rice porous starch obtained with AMG treatment. In contrast, GG tended to decrease the EY in all samples, except in the case of porous corn starches.

This variability in the number of encapsulated cells encountered among starches might be attributed to the different interaction of the coating material with the starch surface (Xing et al., 2015). The porous structure of modified starches could improve the adhesiveness and absorbability of the coating materials (Nagashima, Hirose, & Matsuyama, 2011; Wang, Shao, Wang, & Lu, 2012), but that effect was not related to the pore size or frequency because very weak correlation was observed between EY and pore size ($r = 0.26$; $P < 0.05$) and no correlation between EY and pore frequency. Only in the microcapsules coated with GG, a moderate significant positive correlation was found between EY and the pore size ($r = 0.63$; $P < 0.05$), and pore frequency presented ($r = 0.55$; $P < 0.05$), suggesting that GG accessibility to large pores favors the release of microorganism. Therefore, the effect of coating materials on EY was rather dependent on the starch-coated material interaction, promoting reinforcement of microcapsules or competing with the microorganism for filling the pores.

3.4. Survival of microencapsulated cells under heat treatments

The thermal stability of *L. plantarum* microcapsules was tested at 55 °C (Table 2). Statistical analysis revealed that the type of starch, the enzyme applied to obtain porous starches and the coating material had significant ($P < 0.01$) effects on the cell stability, even after prolonged tests, with the exception of coating materials when 35 min were applied. Previous studies indicate that the survival rate of microencapsulated cells depends on the contact time and temperature gradient (Avila-Reyes et al., 2014), decreasing the survival of *L. plantarum* up to 63% at 55 °C after 10 min (Ashwar et al., 2018). In this study, non-viable cells were detected after heating microencapsulates in native corn starch, while the survival of entrapped cells in native rice starch was approximately 60% after incubation at 55 °C for 20 min, which further decreased till 34% after 35 min at that temperature. The stability conferred by rice starch granules could be understood considering the inclusion of the cells in the interstitial spaces of the aggregates that offers protection against the external environment (Avila-Reyes et al., 2014). In fact, Zhao and Whistler (1994) described the stabilization of food ingredients when those were entrapped within the spherical aggregate of small starch granules, like amaranth starch, rice starch or small wheat starch.

Table 2. Cells viability (%) of *L. plantarum* at 55 °C after 20 and 35 min using native and porous starches as supporting material and either gelatinized starch (GS), guar gum (GG) or xanthan gum (GX) as coating material.

Numbers followed by different letters within a column indicated significant differences.

Starch	Enzyme	Coating	t=20			t=35				
Corn	Native		0.00	±	0.00	a	0.00	±	0.00	a
		S	45.96	±	2.30	cd	0.00	±	0.00	a
		GG	44.94	±	4.07	c	0.00	±	0.00	a
		GX	55.28	±	1.03	fg	0.00	±	0.00	a
	AM		47.53	±	1.88	c-e	43.28	±	1.73	gh
		S	61.39	±	4.69	hi	61.57	±	4.43	k
		GG	82.40	±	3.89	l	52.10	±	0.98	j
		GX	51.40	±	0.54	d-f	39.84	±	1.65	e-g
	AMG		63.95	±	0.98	ij	40.40	±	5.73	fg
		S	52.67	±	10.79	e-g	0.00	±	0.00	a
		GG	57.82	±	5.81	gh	46.78	±	4.54	hi
		GX	33.24	±	1.57	b	26.64	±	3.90	b
Rice	Native		57.17	±	0.08	gh	34.06	±	3.08	cd
		S	63.89	±	2.97	ij	53.16	±	5.32	j
		GG	69.64	±	4.55	j	53.69	±	1.80	j
		GX	56.18	±	1.36	gh	49.87	±	2.92	ij
	AM		51.24	±	1.01	d-f	37.41	±	0.95	d-f
		S	80.32	±	2.54	kl	67.36	±	2.32	l
		GG	76.56	±	3.82	k	37.72	±	0.53	ef
		GX	78.69		0.90	kl	68.62	±	0.76	l
	AMG		55.04	±	1.37	fg	38.84	±	1.96	ef
		S	0.00	±	0.00	a	0.00	±	0.00	a
		GG	54.14	±	2.48	fg	36.02	±	2.36	de
		GX	57.63	±	6.38	gh	31.92	±	4.14	c
P-value	Starch		0.0052			0.0010				
	Enzyme		0.0002			0.0000				
	Coating		0.0027			0.3291				

With regard to porous starches, also a significant effect was observed depending on the supporting material (rice or corn) (Table 2). Encapsulation with corn porous starches conferred stability to the cells, which survived even after 35 min at 55 °C. Conversely,

encapsulates with rice porous starches showed lower stability after 20 min than the one observed when adhering to native starch, but opposite behavior was observed after 35 min heat treatment. According to the investigation of Xing et al. (2014), the collapse of the microcapsules can promote the release of the cells and their subsequent inactivation.

Considering coating, it has been previously reported that multilayers coating enhances the tolerance of cells to thermal treatment (Wang et al., 2012; Xing et al., 2015), thus it would be expected higher survival on the coated encapsulated materials. Nevertheless, significant differences were observed depending on the coating material (Table 2). All coating materials tested allowed maintaining greater number of *L. plantarum* viable cells after 20 min heat treatment when encapsulated in native starches, and even after 35 min in the case of rice supporting material. Consequently, coating materials provided additional protection to the aggregated structure of the rice microcapsules. The protection provided by the coating material varied depending on the characteristics of the porous starch, that is on the enzymatic treatment carried out to obtain the porous starches. Coating did not provide additional protection to the microorganism encapsulated into the starch pores obtained with AMG. Likely, the higher pore size and frequency obtained when starches were treated with AMG could increase the adhesion surface for the cells and the coating material, but the hydrophilic nature of the coating materials could favored the diffusion of hot water molecules through the capsule (Mandal, Hati, Puniya, Khamrui, & Singh, 2014), resulting in large reduction of the probiotic viability. Conversely, immobilized cells in the pores of starches modified with AM exhibited extended thermal resistance, probably the smaller superficial pores covered by coating material provides better fitting for protecting the cells. For this reason, the knowledge of the structures formed after the enzymatic modification of the starch could be taken as a basis for the choice of the coating material. It has been already reported that the survival of microencapsulated bacteria coated with porous starches was higher than that of non-coated microcapsules (Li et al., 2016; Wang et al., 2012; Xing et al., 2015), and that protection is highly dependent on the porous starch concentration (Xing et al., 2014). However, it must be stressed that even when using the same starch concentration, like in the present study, the size and number of pores on the starch surface (Table 1) can significantly affect the stability of the cells. Therefore, the degree of hydrolysis of the starches resulting from the enzymatic modifications may be used for modulating the starch morphology, with subsequent effect on the cells encapsulation and stabilization.

4. Conclusions

The potential of rice and corn starches in their native state or as porous granules to be used as supporting material for obtaining probiotic foods was evaluated using *L. plantarum*. The yield during the encapsulation process indicated the different entrapment undergone by the microorganism depending on the type of starch, being adsorbed on the surface of corn starch but entrapped within the rice granules aggregates. The porosity of the porous starches contributed to increase the encapsulation yield, particularly in the case of rice starch. Coating of the encapsulates provided additional protection, but the effect was dependent on the supporting and coating materials. In general, best encapsulation yield was obtained with encapsulates in porous starches coated with gelatinized starch. Thermal stability of the encapsulates revealed that microencapsulation using porous starches with small pores, like the ones obtained with α -amylase, and coated with gelatinized starch resulted in the highest heat resistance. Microcapsules produced with the mixture of porous starches, obtained with α -amylase, and coating materials can be incorporated in probiotic food formulation to maintain the integrity of the cells.

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