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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 to 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.

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, Rosell, (2018) 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/alginates (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

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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 4000 x *g* for 10 min and re-suspended with sterile peptone water, resulting in a cell suspension containing approximately 2×10^{10} CFU mL^{-1}.

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.

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 nº 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 N_0 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