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Effect of resistant starch and chitosan on survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate

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ABSTRACT

Resistant starch (Hi maize) and chitosan at concentrations of 1% and 0.4% were added to the microencapsulation of *Lactobacillus acidophilus* in alginate beads by extrusion technique. Moist and freeze-dried microparticles were analyzed. The addition of prebiotics and chitosan increased the size of the moist particles, whose diameter was 70.37 μm, while the diameter of the microparticles containing alginate alone was 55.13 μm. In contrast, the freeze-dried microparticles of alginate and alginate + Hi −Maize + chitosan had diameters of 114.51 μm and 112.50 μm, respectively. Both Hi−maize and chitosan provided better protection of probiotics after exposure of the moist microparticles to simulated gastric and intestinal juice, with counts of 6.35 log CFU g^{-1}, while lower counts were observed for the freeze-dried microcapsules. Regarding the viability of the probiotic culture during the storage periods and temperatures, all treatments were viable, with suitable values to confer the probiotic effects (<6 log CFU g^{-1}), with counts up to 6 logs for at least 30 days for the microparticles stored in the freeze-dried form, and 135 days in the moist form, both under storage at room temperature (25 °C).

1. Introduction

According to World Health Organization, probiotics are defined as live microorganisms which when administered in adequate amounts (10^7 CFU g^{-1}) confer health benefits to the host (FAO/WHO, 2001).

In recent years, there is a growing demand for use of probiotics in foods aimed to increase the nutritional and therapeutic value of food products, thus various probiotic strains have been studied and commercially exploited (Franz, 2014).

However, the maintenance of microorganisms viability throughout the product shelf life is a major challenge to the food industry (Douglas & Sanders, 2008), since certain cultures are extremely sensitive to environmental factors such as acidic and oxygen (Kailasapathy & Chin, 2000). The low pH of the stomach together with the presence of bile salts in the small intestine are the main reasons for the dramatic decline in the viability of the probiotic cells after their uptake (Mortazavian & Sohrabvandi, 2007). Therefore, microencapsulation has been widely studied to protect microorganisms from acid environment, bile salts, and oxygen (Oliveira et al., 2007).

Sodium alginate is one of the polymers most used as encapsulating material, since it forms a highly versatile, biocompatible and non-toxic matrix for the protection of active ingredients, especially probiotic microorganisms and cells sensitive to heat, pH, dissolved oxygen, among other factors in which food is exposed during processing and storage (Pasin, Azón, & Garriga, 2012). This polymer is presented as a food additive in the form of white or yellowish brown powder, tasteless and odorless. It is Consisted mainly by the sodium salt of alginic acid, or that is, a mixture of polyuronic acids composed of residues of D-mannuronic and L-guluronic acid (Rowe, 2009).

The microparticles of calcium alginate can be prepared by the extrusion method by dripping a solution of sodium alginate into a solution of a calcium salt, leading to the phenomenon of external ionic gelation (Gombotz and Wu, 1998). In this technique, the

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microorganisms are added to an alginate solution and are immediately incorporated in the form of droplets in a solution of calcium chloride to hardening (Yeo, Baek, & Park, 2001). The interaction of the ions, such as Ca^{2+}, with the carboxyl groups of the polymer chains of the alginate results in the formation of an insoluble gel (Smrdel, Bogataj, Zega, Planinsek, & Mrhar, 2008). In the research conducted by Kim et al. (2008) positive results were obtained for *Lactobacillus acidophilus* ATCC 43121 encapsulated with calcium alginate, by the drip method, during exposure to the *in vitro* gastrointestinal tract and resistance to the thermal treatment.

Although sodium alginate is suitable for encapsulation, its gel is porous and sensible to extreme pH values, thus affecting both the release and protection of the compounds (Mortazavian & Sohrabvandi, 2007). There are several ways to overcome this obstacle and improve stability of microorganisms as, for example, coating the particles with ionic gelling with biopolymers through electrostatic interactions (Patil, Kamalapur, Marapur, & Kadam, 2010) and the addition of prebiotics in the capsule formulation (Chen, Chen, Liu, Lin, & Chiu, 2005).

Lee, Cha, and Park (2004) analyzed the effects of chitosan and alginate microparticles on the survival of *Lactobacillus bulgaricus* KFRI763 in simulated gastric and simulated intestinal juices and on their stability during storage at 4 and 22 °C. Studies conducted by Homayouni (2008), demonstrated that a combination of alginate with starch improves the efficiency of different bacterial cells, particularly lactic acid-producing bacteria, due to the production of granules of good prebiotic structure and effect in the microcapsules.

Therefore, this study aimed to evaluate the effect of resistant starch (Hi–maize) and chitosan on the viability of *L. acidophilus* microencapsulated with sodium alginate against the simulated digestive system and under different storage temperatures.

2. Material and methods

2.1. Inoculum

The probiotic culture *L. acidophilus* La-14 (Danisco) was activated in MRS broth (Himedia) and incubated for 15 h at 37°°C. Then, it was centrifuged at 4670 × g for 15 min and washed with NaCl solution (0.85%). The cells were suspended in saline to obtain a solution containing about 10 log CFU g^{-1}. The concentration of microorganism was adjusted by bacterial growth curve.

2.2. Production of microparticles

Microparticles were produced according to the extrusion technology developed by Liserre, Ré, and Franco (2007), with adaptations. For that, an aerograph (Size of nozzle: 0.3 mm) model EW 110 was coupled to an air compressor Model MB24/BV, on air pressure of 2.72 kgf/cm^2, using the height of 30 cm between the atomizing nozzle and the $CaCl_2$ solution.

The cultures were mixed in two solutions containing 1.0% sodium alginate (Vetec). The first solution contained only sodium alginate (ALG) was sprayed in 0.1 M $CaCl_2$, and the second was composed by sodium alginate + 1% Hi–maize (National Starch), sprayed in 0.1 M $CaCl_2$ containing 0.4% chitosan, as reported by Gaserod, Smidsrod, and Skjakbraek (1998), with adaptations, where 0.4 g of chitosan were dissolved in 90 mL of distilled water acidified with 0.4 mL of glacial acetic acid to achieve a final concentration of 0.4% (w/v). The pH was then adjusted to 5.8 ± 0.2 with 1M NaOH. The mixture was filtered through filter paper and the volume was adjusted to 100 mL. Then, it was autoclaved at 121°°C for 15 min and mixed with calcium chloride solution. The particles were kept under stirring for 30 min in $CaCl_2$ solution, and then removed from the solution using a sieve (50 μm), sterilized, and washed with sterile distilled water.

An amount of moist microparticles was stored in sterile collectors, and the remaining was freeze-dried in Liotop Lyophilizer Model L101 for 24 h.

2.3. Morphological characterization of the microparticles by optical and scanning electron microscopy

Optical microscopy of the moist microparticles was performed using a microscope MDL-150-TPI model, and a digital camera Samsung 14.2 model for image capture. The morphology of the freeze-dried microparticles was evaluated using a scanning electron microscope JEOL brand, model JM6360. The microcapsules were fixed with a double sided tape on aluminum stubs and coated with a thin layer of gold.

2.4. Evaluation of the mean diameter and size distribution of the microparticles

The average size of the moist and freeze-dried microparticles was measured in Mastersizer equipment 2000 (Malvern, Alemanha).

2.5. Viable cells count

Appropriate dilutions were transferred in triplicate to sterile Petri plates, followed by addition of MRS agar (Himedia). Plates were incubated at 37 °C for 72 h in anaerobic jars containing anaerobic generator (Oxoid). The dilution of the microparticles consisted in weighing 1 g of moist microparticles and 0.1g of freeze-dried microparticles, followed by the addition of 9 mL of sterile phosphate buffer solution (pH 7.5) according to the methodology described by Sheu, Marshall, and Heymann (1993).

2.6. Survival of microencapsulated L. acidophilus La-14 under simulated gastrointestinal conditions

This analysis was performed according to the method described by Liserre et al. (2007) with modifications. Aliquots of 1 g of moist microparticles and 0.1 g of freeze-dried microparticles were mixed with 1M HCl pH 1.8, pepsin (pepsin from porcine gastric mucosa P7000, Sigma–Aldrich), and lipase at a concentrations of 3 g L^{-1} and 0.9 mg L^{-1} (lipase from porcine pancreas 62300, Sigma–Aldrich), respectively, prior to incubation at 37°°C under continuous stirring in a refrigerated incubator shaker (model TE-421), for 2 h.

Subsequently, the pH of the samples was adjusted to 5.0. Bile (bovine bile B3883-25G, Sigma–Aldrich) and pancreatin (pancreatin from porcine pancreas P3292, Sigma–Aldrich) was added at a concentration of 1 g L^{-1} and 0.1 g L^{-1}, respectively, and incubated again at 37 °C for 2 h.

Finally, pH was adjusted to 7.5, and the bile and pancreatin concentrations were maintained. The samples were incubated at 37 °C for 2 h under continuous stirring to a total of 6 h of analysis.

Counts were performed after 5, 30, 120, 125, 150, 240, 245, 270, and 360 min of incubation. Serial dilutions were made as described in Section 2.5.

2.7. Viability of the microparticles during storage at different temperatures

Both moist (U) and freeze-dried (L) microparticles were stored at room temperature (25 °C), refrigerated (7 °C), and frozen (−18 °C), of 120 and 60 days respectively.