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Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics

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ABSTRACT

Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit to the host. However, to accomplish this positive influence on Human health, probiotics should survive to the passage through the upper digestive tract in large numbers to unsure a desired beneficial effect in the host. Several encapsulation methods have been used to protect probiotics. Alginate is the most used biopolymer in the production of these systems, although its performance is totally dependent of its structure and chemical characteristics. In this work, alginates with different molecular weights and different mannuronic and guluronic acid residues ratio (M/G ratio) were used in the encapsulation of *Lactococcus lactis* spp. *cremoris* (LLC) aiming the protection of this probiotic bacteria against the harsh conditions of digestion. Alginate-based beads were produced using an external gelation process (extrusion technique) where variables regarding the processing conditions and alginate chemical characteristics were studied to assess their relevance in this process aiming the most efficient encapsulation system. The most important variables influencing the size of alginate beads were the alginate concentration, alginate type (M/G ratio and molecular weight) and the nozzle diameter. Beads with sizes ranged between 1.9 and 3.0 mm were produced using different alginates. Fourier transform infrared (FTIR) spectroscopy showed relevant differences between beads produced proving the impact of different M/G ratios in the beads' chemical structure. In general, low molecular weight and low M/G ratio alginate (Protanal LFR5/60) proved to produce the most well organized (according to SEM analyses), less permeable (pore diameter of 2.52 nm) and stronger alginate beads, moreover molecular weight and M/G ratio proved to be an important variable on the protection of probiotics against the harsh conditions of digestion. Produced beads proved to be efficient in the protection of probiotics (i.e. high viability), with the best performance presented by the medium and low molecular weight alginates.

1. Introduction

Nowadays, probiotics are live microorganisms that when administered in adequate amounts confer a health benefit to the host, being these recognized in several countries (Food and Agriculture Organization of the United Nations/World Health Organization, 2001). Probiotics are able to induce a positive effect on Human health, such as: the production of pathogen inhibitory substances; blocking of pathogenic bacterial cells adhesion sites; nutrient competition and production; degradation of toxins and toxin receptors; and the modulation of the immune responses (Prakash, Tomaro-Duchesneau, Saha, & Cantor, 2011). Probiotics have already presented some positive effects on human health, such as: the reduction of the expression of some biomarkers responsible for colonic cancer; treatment and prevention of acute diarrhoea in children; prevention of an initial attack of pouchitis, maintaining remission of ulcerative colitis; to alleviate symptoms in persons with functional abdominal pain; improve lactose digestion and reduce symptoms related to lactose intolerance; and to reduce the risk of necrotizing enterocolitis (Aureli et al., 2011;

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Sullivan & Nord, 2005).

However, to accomplish this positive influence on Human health, probiotics should survive to the passage through the upper digestive tract in large numbers to ensure a desired beneficial effects in the host (Gilliland, 1989). The minimum number of viable cells suggested to achieve the benefits mentioned before is in the range of 10^8-10^9 viable cells per day/dose (Hou, Lin, Wang, & Tzen, 2003; Doleyres & Lacroix, 2005). Considering the limitations of free probiotics survival during digestion, microencapsulation is generally seen as a simple and efficient solution to improve probiotics survival during this process. The most common techniques used in the encapsulation of probiotics are extrusion, emulsification and spray-drying (Kailasapathy, 2009; Tripathi & Giri, 2014). Extrusion has been the most used technique on the microencapsulation of probiotics, due to its simplicity of operation, good performance of the process in a laboratorial environment, lower cost, and assurance of a high cell viability (de Vos, Faas, Spasojevic, & Sikkema, 2010). An important issue in probiotics encapsulation is the size of the produced capsules, because of its influence in probiotics protection and into alterations on food eating sensation, changing the organoleptic properties of foods. The main variables that influence the capsules' size in extrusion technique are: the concentration of the polymer and cross-linker solutions; the flow-rate of the dropping solution; the distance from the needle to the cross-link solution; and the nozzle size (Brun-Graeppi et al. 2011). To create the main structure of these capsules, divalent calcium ions (Ca^{2+}) are commonly used, although other ions can also be applied (Tam et al., 2011). Regarding the different polymers used, alginate is the most applied material to capsules formation, due to its low price, facility to gel formation and biocompatibility (Chen, Wang, Sánchez-Soto, Schiraldi, & a, 2012; Klein, Stock, & Vorlop, 1983, pp. 86–91; Quong, Neufeld, Skjåk-Braek, & Poncelet, 1998; Smidsrd & Skjak-Brae, 1990; Tanaka & Matsumura, 1983). Alginate is a polysaccharide extracted from brown algae and is composed of randomly 1–4 linked β-D-mannuronic acid and α-L-guluronic acid, M blocks and G blocks, respectively (Smidsrd & Skjak-Brae, 1990). Alginate composition changes depending of its molecular weight (MW) and the ratio between M and G blocks (M/G ratio), that leads to alginates with different characteristics when crosslinked with calcium ions. More specifically, G blocks have more affinity to calcium ions than M blocks. These characteristics are able to influence the structure of the capsule, thus creating different capsules considering their permeability to low molecular weight compounds. In one hand, alginates with a higher M/G ratio are capable to create more permeable alginate gel matrices (Khanna, Moya, Opara, & Brey, 2010) and in the other hand alginates with a lower M/G ratio lead to stronger structures due to the bigger affinity of the G blocks with calcium ions, compared to M blocks (Sarmento, Ribeiro, Veiga, Ferreira, & Neufeld, 2007). As mentioned before alginate has been used in several works to protect probiotics using different alginates (Barbosa & Teixeira, 2016; Smidsrd & Skjak-Brae, 1990). However, in general these works do not focus in using different types of alginate. Even so, Mandal, Puniya, and Singh (2006) studied how alginate concentration could influence the survival of *Lactobacillus casei* NCDC-298, concluding that the survival increased proportionally with alginate's concentration. There are some works where alginates with different characteristics are used although the structures produced are not applied in probiotics protection (Bajpai & Sharma, 2004; Klein et al., 1983, pp. 86–91). Therefore, there is a lack of information about which alginate characteristics (MW and M/G ratio) suits better the protection of probiotics considering the harsh conditions of digestion.

This work evaluates how the different variables during extrusion influenced beads' size and how alginates with different molecular weight and M/G ratio influence the beads' size, porosity, and their capacity to protect probiotics against the harsh conditions of digestion.

2. Methods and materials

2.1. Materials

Sodium alginate Protanal CR8133 (M/G = 65/35, MW = 90–180 kDa), Protanal CR8223 (M/G = 65/35, MW = 250–350 kDa) and Protanal LFR5/60 (M/G = 30/70, MW = 20–60 kDa) were kindly given by FMC BioPolymer (Brussels, Belgium) and presented a viscosity for a 1% solution at 20 °C and at 30 rpm of 39 mPa s, 326 mPa s and 7 mPa s, respectively, measured with a rotational viscometer (Model ELV-8, Viscometers U.K. Ltd, London, U.K.). Calcium chloride ($CaCl_2$) was purchased from Panreac (Barcelona, Spain). M17 broth was purchased from Oxoid (Hampshire, England). M17 agar was purchased from Merck (Munich, Germany). Potassium chloride (KCl), monopotassium phosphate (KH_2PO_4), sodium bicarbonate ($NaHCO_3$), sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2(H_2O)_6$), ammonium carbonate ($(NH_4)_2CO_3$), calcium chloride dihydrate ($CaCl_2(H_2O)_2$), sulfuric acid (H_2SO_4), L-lactic acid ($C_3H_6O_3$), hydrogen chloride (HCl), sodium citrate ($Na_3C_6H_5O_7$), phosphate-buffered saline (PBS), were purchased from Sigma-Aldrich (St. Louis, USA). To perform the gastrointestinal simulations pepsin (from porcine, car nº SLBL2143V, 3616 $U.mg^{-1}$), pancreatin (from porcine, cat nº SLBL3953V, 6.1 $U.mg^{-1}$) and bile salts (from porcine, cat nº SLBK9078V, 164 mM) were purchased from Sigma-Aldrich. The probiotic used in this work, *Lactococcus lactis* ssp. *cremoris* SK 110 (LLC), was obtained from Nizo (Nizo Food Research, Ede, The Netherlands).

2.2. Preparation of alginate beads by external extrusion

Alginate beads production were studied based on two experimental designs where external and internal parameters were evaluated separately (see section 2.12 Statistical analysis). Briefly, a volume of 10 mL of alginate solution (with the concentration tested in each experiment), was dropped in 90 mL of a solution of $CaCl_2$, with different concentrations. After that the alginate solution was transferred to a syringe, and dropwise, with the help of a syringe pump, at different flow rates, through needles with different diameters, into the $CaCl_2$ solution that was placed at variable distances, and magnetically stirred (with different stirring speeds). After the sodium alginate solution had been extruded into the $CaCl_2$ solution, the solution continued to be magnetically stirred for 20 min, in order to allow alginate beads to harden. Afterwards, the alginate beads were recovered by a sieve and used as it or freeze-dried.

In Experiment a), external variables were studied such as: flow rate (1, 3 and 5 mL min^{-1}), needle-$CaCl_2$ solution distance (1, 5 and 10 cm) and the stirring speed (60, 100 and 300 rpm). The following internal parameters were maintained: 2% (w/v) of sodium alginate Protanal CR8133 solution, a 0.8 mm needle diameter and a 0.1 $mol.L^{-1}$ $CaCl_2$ solution. In Experiment b), internal variables were studied such as: needle diameter (0.3; 0.6 and 0.9 mm), alginate Protanal CR8133 concentration (1, 2 and 3% w/v) and $CaCl_2$ concentration (0.25, 0.5 and 1 $mol.L^{-1}$). For Experiment b) and c) the following conditions were maintained: 300 rpm, 1 mL min^{-1} and 1 cm of distance. To the last test, where the three different alginates were tested, the conditions used were: 0.3 mm needle diameter, a 1 $mol.L^{-1}$ $CaCl_2$ concentration and a 1% (w/v) of sodium alginate solution was used in order to guarantee the spherical shape and uniformity of the beads, being the other variables maintained as referred before.

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