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Effect of starch filler on calcium-alginate hydrogels loaded with yerba mate antioxidants

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ABSTRACT

A liquid extract of yerba mate (*Ilex paraguariensis*), with antioxidant properties was encapsulated in calcium-alginate hydrogels containing corn starch as filler at different concentrations. Hydrogel beads were characterized for morphological and size aspects, encapsulation efficiency, Fourier Transform Infrared Spectrometry (FT-IR) and thermal behavior. Addition of starch improved the encapsulation efficiency from 55 to 65%. In vitro release of polyphenols was analyzed in model gastric and intestinal media. The recovery of encapsulated polyphenols occurred mainly in the simulated gastric fluid (85%). Kinetics and release mechanism were satisfactorily fitted to semi-empirical models. The incorporation of starch filler (2 g/100 mL) in calcium alginate hydrogels modified the release profile of polyphenols in acidic medium. In calcium alginate beads, a release mechanism combining erosion and diffusion was observed. Whereas, for polyphenols release of starch loaded beads, only diffusion was relevant.

1. Introduction

Application of hydrogels obtained from polysaccharides in the food industry are constantly increasing, due to the high demand of natural and environmentally compatible materials (Farris, Schaich, Liu, Piergiovanni, & Yam, 2009). In this way, hydrogels have been used as carriers of bioactive compounds such as natural antioxidants, cells, unsatured oils, drugs, among others (Deladino, Anbinder, Navarro, & Martino, 2008; Gbassi, Vandamme, Ennahar, & Marchioni, 2009; Goh, Heng, & Chan, 2012; Pongjanyakul & Rongthong, 2010).

Sodium alginate is soluble in water and can form hydrogel beads by dropping the aqueous solution into a divalent or polyvalent cation solution (Draget, Steinsvåg, Onsøyen, & Smidsrød, 1998). Although this is a simple and fast way of obtaining encapsulating systems, the method presents a major limitation consisting in loss during bead preparation. Active compound losses are favor by both, the time necessary for the cation to diffuse into the bead and the compound concentration gradient between the beads and surrounding solution. Besides, the presence of macropores in the alginate matrix facilitates the diffusion of hydrophilic molecules (George & Abraham, 2006; Gouin, 2004). However, some researchers were able to solve this problem by mixing alginate with other polymers such as starch, chitosan, cellulose, pectin, among others. In some cases, mechanical and physical properties of beads have been improved, as well (Chan et al., 2011; Santagapita, Mazzobre, & Buera, 2012). Other authors employed ionic gelation to encapsulate active compounds by other techniques like internal gelation or absorption method. A promising alternative was proposed by Chan, Yim, Phan, Mansa, and Ravindra (2010). They encapsulated an herbal aqueous extract in calcium alginate beads using the absorption method, and found that this technique gave a 2–6 times higher encapsulation efficiency than the direct extrusion method. Stojanovic et al. (2012) used electrostatic extrusion and improved the encapsulation loading of thyme extract by about 16% in comparison to the absorption method. They eliminated the driving force for diffusion by maintaining the same concentration of polyphenolic compounds in the gelling solution as within the gel matrix. The incorporation of a filler material into alginate matrix is another strategy for solving the above mentioned disadvantages. Starch has been employed to increase the probiotic bacteria entrapment (Sultana et al., 2000). Rassis, Saguy, and Nussinovitch (2002) found that values between 10 and 30% of corn starch reduced calcium alginate shrinkage.

Yerba Mate dried and minced leaves are used to prepare a highly consumed tea-like beverage, mainly in Argentina, Brazil, Uruguay and Paraguay. At present, almost one hundred scientific works are claiming its health benefits (Sciencedirect, 2012), most of them related with the antioxidant properties attributed to the high polyphenol content of aqueous yerba mate extracts (Anesini, Turner, Cogoi, & Filip, 2012; Bastos et al., 2007; Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2011; González de Mejía, Song, Heck, & Ramirez-Mares, 2009).

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As suggested by Moure et al. (2001) the antioxidant compounds from natural sources could be used to increase the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. The addition of antioxidants is required to preserve flavour and colour and to avoid vitamin destruction. However, the stability, bioactivity, bioavailability and unpleasant taste of most phenolic compounds, limit their applications (Fang & Bhandari, 2010; Munin & Edwards-Lévy, 2011). In this work, the encapsulation of yerba mate polyphenols, instead of using the free compounds, is proposed as an alternative to alleviate these deficiencies. In previous studies, we developed and characterized yerba mate capsules of calcium alginate with and without chitosan external layer (Anbinder, Deladino, Navarro, Amalvy, & Martino, 2011; Deladino et al., 2008). In the present one, we used a different strategy to increase the loading efficiency and to modulate its release. In this sense, the aim of this work was to study the effect of incorporation of corn starch granules to calcium alginate hydrogels, as a filling agent, to prevent yerba extract loses during bead formation and handling, and to study its release mechanism.

2. Materials and methods

2.1. Yerba mate extract

Extracts were obtained from commercial yerba mate (YM), *Ilex paraguariensis*, samples ("*La Merced*, de campo", Las Marías, Corrientes, Argentina); 3 g of YM with 100 mL of distilled water were placed in a thermostatic bath (Viking, Argentina) at 100 °C for 40 min. Once obtained, the extracts were filtered, cooled in an ice bath and kept in dark flasks until further use (Deladino et al., 2008).

Total polyphenol content was determined by the Folin-Ciocalteu method (Schlesier, Harwat, Böhm, & Bitsch, 2002). Briefly, 2 mL of Na_2CO_3 (2 g/100 mL) (Anedra, Argentina) were mixed with 200 μL of the sample and 200 μL of Folin-Ciocalteau reagent (Anedra, Argentina, 1:1 diluted). After 30 min, sample absorbance was measured at 725 nm in a spectrophotometer (Shimadzu, UV-mini 1240, Japan). Chlorogenic acid (Fluka, USA) was used as standard. Results were expressed as mg standard equivalent/g of YM dried leaves.

2.2. Preparation of alginate hydrogels beads

The hydrogel beads were prepared by ionic gelation obtaining a calcium alginate matrix. The sodium alginate (Sigma–Aldrich, USA) was dissolved in the YM extract (3 g/100 mL). Once homogenized and sonicated, the solution was forced with a peristaltic pump at 45 rpm (Gilson Minipuls 3, France) into a syringe (diameter: 1 mm) to drop into a calcium chloride solution (0.05 mol/L). The beads were maintained in the gelling bath to harden for 15 min. Then, they were filtered and washed with buffer solution (acetic-acetate, pH 5.5). These hydrogel beads containing YM will be referred as CA (Calcium alginate hydrogels).

Calcium alginate-starch hydrogels (CAS) were obtained by adding different concentrations of commercial corn starch (Maizena®) (0.1, 0.5, 1, 2, 5 and 10 g) into 100 mL of the YM-alginate solution described previously.

2.3. Hydrogels characterization

2.3.1. Encapsulation efficiency and loading capacity

Encapsulation efficiency of hydrogels beads (%EE) was calculated with the following equation:

$$\%EE = \left(\frac{m_\infty}{m_e}\right) \times 100 \quad (1)$$

where m_∞ is the amount of YM polyphenols determined in sodium citrate solution and m_e is the amount of YM polyphenols of the extract employed in beads formulation. The value of m_∞ was determined adding a known amount of beads to a sodium citrate solution (5 g/100 mL). Beads were placed in an orbital shaker (Orbit Environ Shaker, Lab Instruments, USA) at 37 °C and 180 rpm for 3 h. Total polyphenol mass was quantified by Folin-Ciocalteau method described in Section 2.1.

The loading capacity (L) was obtained with following equation:

$$L = \left(\frac{m_\infty}{m_b}\right) \quad (2)$$

where m_b is the mass of dried beads employed in the assay. Results of loading capacity were expressed in mg of chlorogenic acid/g beads (dried basis).

2.3.2. Morphology and size of hydrogel beads

The mean size of beads was calculated analyzing photographs of at least 50 beads with the ImageJ processing software (Schneider, Rasband, & Eliceiri, 2012). Photographs were acquired in a stereomicroscope Leica MZ 10F (Germany), equipped with a camera (Leica DFC 490, Germany).

The shape of the hydrogels was characterized using the sphericity factor (SF), which is calculated by the following equation (Chan, Lee, Ravindra, & Poncelet, 2009):

$$SF = \frac{d_{\max} - d_{\min}}{d_{\max} + d_{\min}} \quad (3)$$

where d_{max} is the largest diameter and d_{min} is the smallest diameter perpendicular to $d_{\max}$. The d_{max} and d_{min} were measured using the ImageJ processing software. The SF varies from 0 for a perfect sphere to approaching unity for an elongated object.

Scanning electronic microscopy (SEM) analysis was performed using a FEI, Quanta 200 microscope (Netherlands). Dried beads were attached to stubs using a two-sided adhesive tape, then coated with a layer of gold (40–50 nm) and examined using an acceleration voltage of 20 kV. Hydrogel beads were dried in an air convection oven at 65 °C for 3 h for SEM, DSC and FT-IR studies.

2.3.3. Humidity content, water activity and bulk density

Humidity content (%) was measured gravimetrically, drying the beads in an oven at 105 °C until constant weight (AOAC, 1998). Values of water activity (a_w) were determined using an AquaLab Serie 3 TE (USA) apparatus (AOAC, 1998). The bulk density (ρ_B) of the beads was determined by pouring a known mass of beads into a measuring cylinder, and it was calculated by dividing the mass by the bulk volume (Abdullah & Geldart, 1999).

2.3.4. Antioxidant activity of free and encapsulated yerba mate extract

Antioxidant activity of YM extract was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) (Sigma–Aldrich, USA) as a free radical, according to the method described by Brand-Williams, Cuvelier, and Berset (1995). Different concentrations of extract were tested (0.32–3.0 mg yerba mate extract/mL). A solution of 25 mg DPPH•/L of ethanol was prepared. One hundred μL of each sample was mixed with 3.9 mL of DPPH• -ethanol solution. Absorbance was determined at 517 nm until the reaction reached a plateau. These values and the initial DPPH• ones were used to calculate the percentage of DPPH• remaining at the steady state. These percentages were plotted against yerba mate extract concentration values. From this plot, antiradical activity was calculated as the amount of yerba mate extract needed to decrease the initial DPPH• concentration by 50%. This value is commonly expressed as Efficient Concentration (EC_{50}), in mg antioxidant per mg DPPH•.