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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•.

To analyze the antioxidant activity of extract once encapsulated, a known amount of beads with an appropriate volume of distilled water were placed in an orbital shaker (Orbit Environ Shaker, Lab Instruments, USA) during 20 h, at 37 °C and 180 rpm. The antioxidant activity of the released extract was determined as previously described, and was expressed as the percentage of remaining DPPH•.

2.3.5. Differential scanning calorimetry (DSC)

Thermal analysis was performed on the sodium alginate, lyophilized YM extract and dried beads without and with YM extract. The equipment used was a DSC Q100 (TA Instruments, USA), calibrated with an Indium standard. Samples of 3–5 mg were placed in aluminum pans hermetically sealed and an empty pan was used as the reference. Samples were heated from 25 °C to 300 °C with at a heating rate of 10 °C/min.

2.3.6. Fourier transform infrared spectrometry (FT-IR)

The employed equipment was a Nicolet IS-10 (Thermo Scientific, EEUU) and the spectral analysis was performed with the sotfware Omnic version 8.1. (Thermo Scientific, Inc, EEUU). Disks (7 mm) were obtained by milling 1 mg of sample with 100 mg of KBr and were analyzed under transmission mode, taking 64 scans per experiment with a resolution of 4 cm^{-1}. Samples of lyophilized yerba mate extract, CA beads without and with YM extract and CAS beads with YM extract were analyzed.

2.4. Analysis of yerba mate extract release in simulated digestive fluids

2.4.1. Release kinetics of yerba mate polyphenols

For in vitro release studies a modified method from "Delayed release dosage forms. Method B" of US Pharmacopeia was performed (USP, 2011). Chlorhydric acid (Anedra, Argentina) 0.1 mol/L was used as Simulated Gastric Fluid (SGF). The pH of the solution was adjusted to pH = 2 with NaOH 1 N. Simulated intestinal fluid (SIF) was Sorensen's phosphate buffer with a pH of 7.4.

An Erlenmeyer containing a known amount of beads with 25 mL of SGF was placed in an orbital shaker at 37 °C and 180 rpm. Aliquots of 200 μL of supernatant were removed at different times for 3 hours. After this period, capsules were filtered and placed in another Erlenmeyer with 25 mL of SIF for 2 h.

Total polyphenol content was determined as described previously (Section 2.1). Assays were performed at least three times. The percentage of released extract was calculated as follows:

$$\text{Released extract}\,(\%) : \frac{m_t}{m_\infty} \times 100 \tag{4}$$

where m_t is the mass of polyphenols quantified at each time t, m_∞ is the mass of yerba mate polyphenols loaded in the capsules determined experimentally in sodium citrate solution.

Based on Ritger and Peppas (1987) studies, an empirical model was applied to determine the release mechanism as follows:

$$\frac{m_t}{m_\infty} = k \cdot t^n \tag{5}$$

where k is a constant incorporating characteristics of the macromolecular network system and n is the diffusion release exponent, commonly used to characterize the type of active agent transport. This model is valid only for the $m_t/m_\infty < 0.6$.

2.4.2. Weight change of hydrogel beads in acidic medium

A similar assay to that described in section 2.4.1 was performed. Different vessels with equal amount of beads and gastric fluid were prepared for each time. The beads were removed from the solution at different times, blotted with filter paper Whatman # 1, and weighed immediately. The kinetics of weight change (ΔW %) of hydrogels beads was calculated with the following equation:

$$\Delta W(\%) = \left(\frac{W_t - W_0}{W_0}\right) \times 100 \tag{6}$$

where W_0 and W_t are the weight of the beads at time 0 and t, respectively. All the experiments were performed in triplicate.

Also the change in the average size during the immersion in SGF was photographically monitored using the Leica stereomicroscope, and the mean diameters of the hydrogels were calculated.

2.5. Statistical analysis

Data analysis was performed with the software SYSTAT INC. (Evenston, USA) and DDSolver software (Zhang et al., 2010). Analysis of variance (ANOVA) and means comparison were carried out. Also, non-lineal model was used for fitting release data. Unless indicated, a level of 95% of confidence ($\alpha = 0.05$) was used.

3. Results

3.1. Role of starch filler in the encapsulation efficiency of YM polyphenols

Fig. 1 shows encapsulation efficiency of CA and CAS systems containing different concentrations of starch filler. The initial polyphenol content of the extract was 127 ± 1.16 mg chlorogenic acid/g YM dried leaves. The control system (CA) entrapped the 55% of these polyphenols (Fig. 1). ANOVA showed that the addition of starch was a significant factor for the efficiency of YM extract encapsulation. Encapsulation efficiency increased to 65% with the addition of starch at 2% to the alginate matrix (CAS). No significant differences in efficiency were found between concentrations of 1 and 2% of starch. CAS hydrogels with starch concentrations of 0.1 and 0.5% did not showed significant difference respect to CA hydrogels. The addition of filler above 2% (data not shown) did not improve the efficiency of encapsulation of polyphenols of yerba mate. Besides, these solutions were of difficult handling due to its high viscosity. The effect of starch filler on improving the entrapment capacity of calcium alginate hydrogels has been reported in earlier studies (Rassis et al., 2002; Sultana et al., 2000). Thus, the CAS hydrogels with 2% starch were selected to carry on characterization experiments.

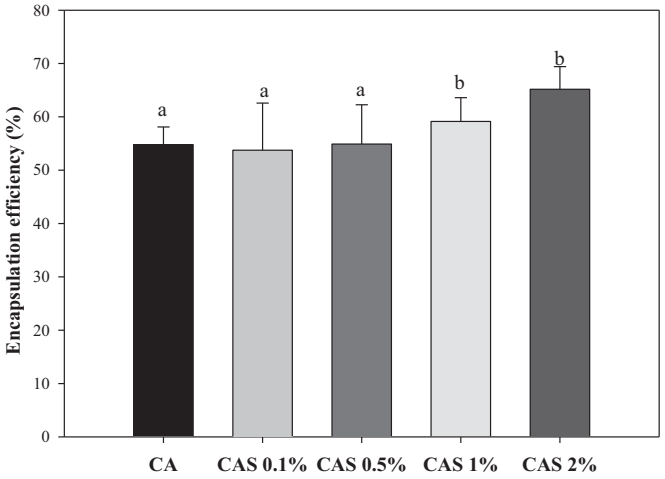

Fig. 1. Encapsulation efficiency of calcium alginate (CA) and calcium alginate-starch (CAS) hydrogels.

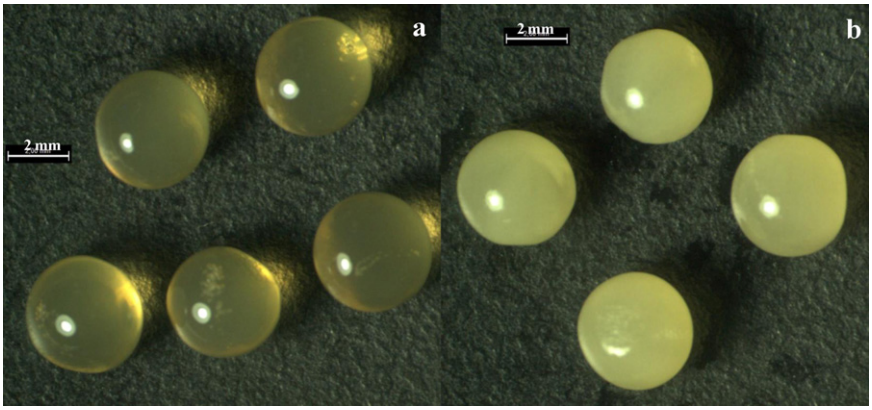

Fig. 2. Yerba mate calcium alginate beads (a) and starch filled calcium alginate beads (b).

The loading capacity (L) was 17.3 and 19.5 mg of chlorogenic acid/g beads (dried basis), for CA and CAS hydrogels, respectively. The higher amount of L for CAS hydrogels is in agreement with the encapsulation efficiency. This magnitude helps calculating the amount of beads necessary to reach a determined antioxidant level in a food formulation.

3.2. Morphological analysis and physical properties of alginate hydrogels beads

Optical micrographs of calcium alginate (CA) and calcium alginate-starch (CAS) hydrogels beads loaded with YM extract are shown in Fig. 2. The microspheres showed a homogeneous size distribution with an average diameter of around 3.7 cm (Fig. 2). As shown in Table 1, starch addition did not affected the average diameter, sphericity factor, bulk density, moisture content and water activity with respect to calcium alginate beads. Chan et al., 2009 investigated the influence of operating conditions on the morphological characteristics of the alginate beads. They suggested that systems with $SF < 0.05$, can be considered spherical. Therefore, beads obtained in this study corresponded to a high degree of sphericity (Table 1). The homogenous size distribution and the high degree of sphericity of hydrogels demonstrated that the operating conditions during ionic gelation were adequate and under control.

SEM micrographs revealed the internal structure of hydrogels beads (Fig. 3). Calcium alginate hydrogels showed some cracks on the surface, similar to those observed by Fundueanu, Nastruzzi, Carpov, Desbrieres, and Rinaudo (1999) (Fig. 3a and b). The internal structure showed layers which were associated to radial gelation from the surface toward the inner core of the bead. Chan et al. (2011) using X-ray and SEM images of lyophilized beads, found evidences of the external gelation process that take place during crosslinking of sodium alginate with calcium chloride solutions.

When the starch was added, the internal and external structures of the beads changed since the filler partially occupied the interstitial spaces of the system (Fig. 3c and d). Starch granules were homogeneously distributed within the beads, what highly contributed to maintain the spherical shape of dried beads.

3.3. Antioxidant activity of free and encapsulated yerba mate extract

EC_{50} value of YM extract was 0.122 mg of chlorogenic acid equivalent/mg DPPH•, this value denotes a high antioxidant capacity according to previous works (Deladino et al., 2008). Among the main bioactive compounds present in yerba mate, chlorogenic acid showed the highest contribution (Anesini et al., 2012). Also, the antioxidant capacity of this extract has been compared with other natural substances (ascorbic, chlorogenic and gallic acids) and synthetic additives (BHT and BHA) obtaining similar results (Bastos et al., 2007; González de Mejía et al., 2009).

To determine if the encapsulation process modified the antioxidant activity of the YM extract, the corresponding value for the free extract was considered as the maximum possible activity. Once the encapsulated extract was released from both CA and CAS systems, the antioxidant capacity was measured. No significant differences in % remaining DPPH• were found between encapsulated YM extract and the same amount of free extract. Starch addition did not modify antioxidant properties of encapsulated extract, since the two types of hydrogels (CA and CAS) gave similar % remaining DPPH• values ($p < 0.05$).

3.4. Thermal behavior of alginate hydrogels (DSC)

Fig. 4 shows the DSC thermograms of lyophilized yerba mate extract (curve A), sodium alginate (curve B) and beads without and with YM extract CA (curve C–D) and CAS (curve E–F), respectively. The YM extract showed only an endothermic transition at 85 °C (curve A), which is very broad probably due to the great diversity of compounds present in the extract.

Sodium alginate showed an exothermic decomposition peak at 225 °C (Curve B). Similar peaks were obtained by Sarmento, Ferreira, Veiga, and Ribeiro (2006) and Anbinder et al. (2011), at temperatures of 240 and 247 °C, respectively. However in calcium alginate beads (curve C–F), the decomposition peak characteristic

Table 1
Physical properties of hydrogels beads.

Hydrogel formulation	Average diameter (mm)	SF	ρ_B (g/mL)	a_w	Moisture content (%)
CA	3.88 ± 0.23	0.04 ± 0.01	0.78 ± 0.03	0.98 ± 0.00	96.66 ± 0.13
CAS	3.69 ± 0.21	0.03 ± 0.01	0.79 ± 0.03	0.98 ± 0.01	94.81 ± 0.11

CA: calcium alginate beads; CAS: calcium alginate-starch beads.

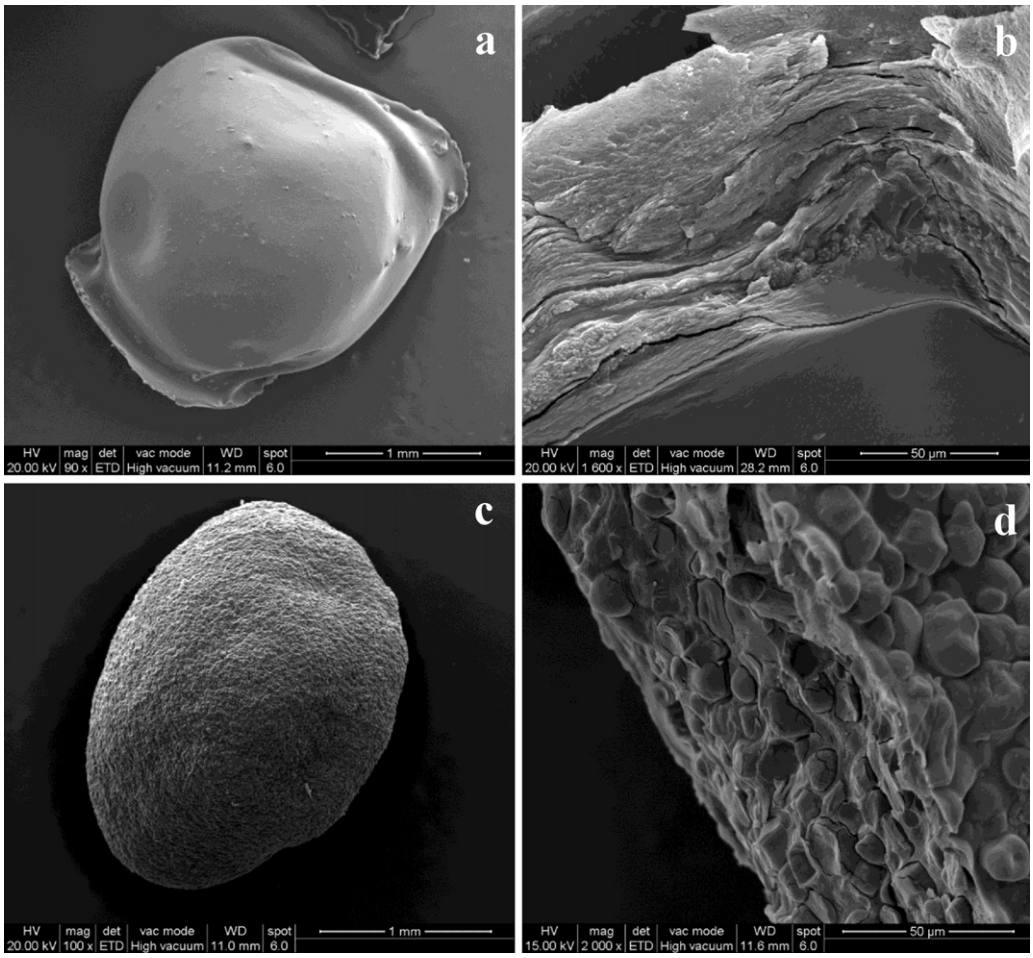

Fig. 3. Scanning electron micrographs of calcium alginate beads (a and b) and starch filled calcium alginate beads (c and d).

of sodium alginate was not observed due to the formation of the structure of "egg box" with calcium ions.

Regarding CA beads without and with YM extract, endothermic peak at 194 and 198 °C, respectively were obtained. According to Sarmento et al. (2006) the endothermic peaks are correlated with the loss of water associated with hydrophilic groups of this polymer.

CAS beads without and with YM extract (curve E–F), showed endothermic peaks that shifted to 209 and 201 °C, respectively. The peak shift towards higher temperatures was attributed to reinforcement in alginate matrix with the addition of starch filler.

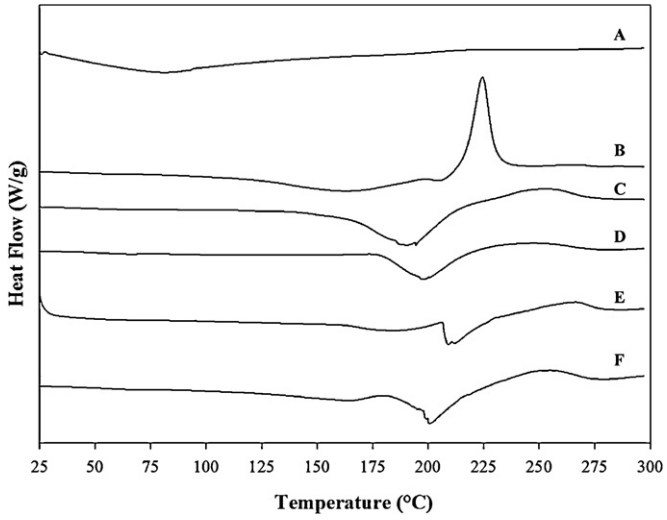

Fig. 4. DSC thermographs obtained for lyophilized YM extract (curve A), sodium alginate (curve B), CA beads without and with YM extract (curve C–D) and CAS beads without and with YM extract (curve E–F).

3.5. FT-IR studies

Fig. 5 shows IR spectra of yerba mate extract and capsules with and without antioxidant extract. Yerba mate extract (Curve A) presented bands characteristic of phenolic compounds, as described in an earlier work (Anbinder et al., 2011). A broad band centered around 3380 cm^{-1} assigned to the stretching modes of O—H groups and a shoulder at 3218 cm^{-1} that corresponds to the hydroxyl groups involved in an intermolecular hydrogen bond were observed. Besides, peaks at 1520 cm^{-1} attributed to the C=C vibration of an aromatic ring were also seen, as well as the C—O stretching band at 1270 cm^{-1}.

FT-IR spectrum of CA hydrogels containing yerba mate extract (Curve C) showed a shoulder at 1520 cm^{-1} (C=C vibration), typical of phenolic compounds of YM extract, that was not identified in CA hydrogels without YM spectrum (Curve B). Anbinder et al. (2011) analyzed the FT-IR spectra of calcium alginate beads with and witout chitosan layer containing YM extract and found relevant changes in 1570–1540 cm^{-1} region. They attributed this to the interaction between the polar groups of the active compounds and the hydroxilic or carboxilic groups of the polymers. With the

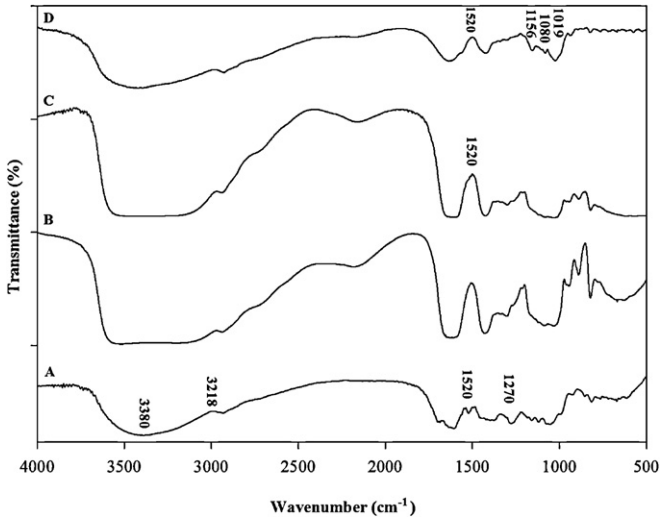

Fig. 5. FT-IR spectra of lyophilized YM extract (curve A), CA beads without and with YM extract (curve B–C) and CAS bead with YM extract (curve D).

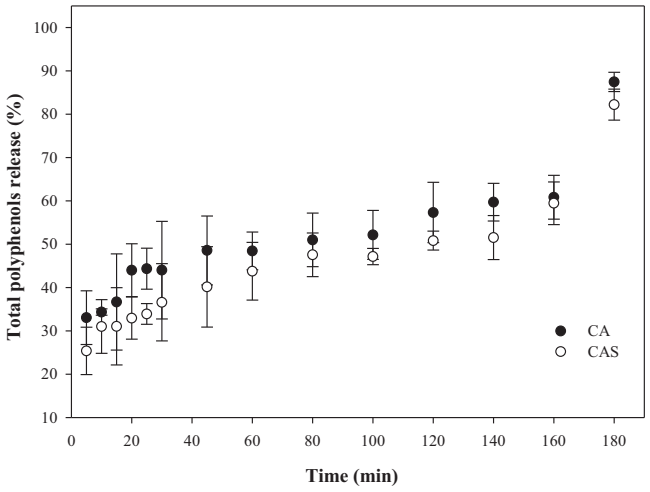

Fig. 6. Release kinetics of yerba mate antioxidants in simulated gastric fluid.

addition of the filler to alginate matrix, IR spectra showed changes in the region between 1500 and 900 cm^{-1} (Curve D). Signals around 1156, 1080 and 1019 cm^{-1} were attributed to vibrations of C—O and C—C of glucose units of starch polymer. The band located at 3390 cm^{-1} corresponds to the stretching of—OH groups. These results agree with those found in literature (Błaszczak, Valverde, & Fornal, 2005; Kizil, Irudayaraj, & Seetharaman, 2002; Popov & Lewin, 1996).

3.6. Kinetics and mechanism release of YM polyphenols in simulated digestion

The release of YM polyphenols in gastric and intestinal simulated fluids was analyzed. A high percentage (around 85%) of recovery of YM polyphenols was obtained in SGF at 180 min of assay, for both hydrogels formulations (CA and CAS). The remaining content of encapsulated polyphenols was released at 30 min of immersion in SIF, thus the encapsulated YM polyphenols were not affected under these conditions. Accordingly, Bermúdez-Soto, Tomás-Barberán, and García-Conesa (2007) and Tagliazucchi, Verzelloni, Bertolini, and Conte, (2010) stressed that acidic media did not affect polyphenol compounds of chokeberry. Moreover, several researchers found no effect of enzymes on tea polyphenols during gastric digestion (Green, Murphy, Schulz, Watkins, & Ferruzzi, 2007). Thus, the initial load of yerba mate polyphenols could reach the gut without modifications.

The release kinetics of YM polyphenols in SGF is shown in Fig. 6, a gradual release up to 160 min for CA and CAS hydrogels, with a lower rate for the beads with 2% starch filler was observed. The time required to reach the 50% (T_{50}) of polyphenols in the external medium was used to evaluate the release rate from hydrogel. The values were extrapolated from Fig. 6, being CAS hydrogel higher than that of CA (100 and 60 min, respectively). This difference was attributed to the presence of starch granules within the calcium alginate matrix (Fig. 3). Several authors reported the use of fillers as barrier to solvent flow, delaying active compound release (Gal & Nussinovitch, 2007; Roy, Bajpai, & Bajpai, 2009; Singh, Sharma, Kumar, & Gupta, 2009).

Ritger and Peppas model was fitted to experimental data to analyze the release mechanism. A nonlinear least-squares regression was applied using the DDSolver software (Zhang et al., 2010). The kinetic parameters obtained are shown in Table 2. The goodness of fit was evaluated using the corrected coefficient of correlation (R^2_{cor}) and the residual analysis.

This model showed good fitting with high values of R^2_{cor} and a good residual distribution. As mentioned above n is the diffusion release exponent that could be used to characterize the different release mechanism: $n = 0.5$ (Fickian diffusion), $0.5 < n < 1.0$ (anomalous transport), $n = 1.0$ (case II transport) and $n > 1.0$ (super case II transport) (Pothakamury & Barbosa-Cánovas, 1995). In this study for both hydrogels formulations, diffusional exponent (n) lower than 0.5 were obtained (Table 2). Similar values were attributed to diffusion mechanism by others authors (Ades, Ungar, & Shimoni, 2012; Heinemann, Zinsli, Renggli, Escher, & Conde-Petit, 2005).

Besides, hydrogels showed a size decrease and a weight loss during release assays in SGF and were totally disintegrated by the immersion in SIF. Fig. 7 shows the percentage of weight change and the average diameter variation of the hydrogels at pH 2, with exposure time. After passing SGF solution, hydrogel beads remained spherical but their initial size (3.7 mm) was reduced throughout the assay. The characteristic green color of yerba mate extract gradually disappeared to show a translucent appearance for CA hydrogels and whiteness for CAS ones. In digestion process the hydrogels can exhibit changes in size and morphology. According to Lao, Peppas, Boey, and Venkatraman (2011) degradation refers to the actual process of polymer chain cleavage/bond hydrolysis into shorter chains or oligomers, while erosion refers to mass loss from the matrix, which may include the loss of (water-soluble) monomers, oligomers and/or other degradation products. Based on this statement, the large reduction in size of the hydrogels observed in the first minutes of immersion in SGF (0–10 min), could be attributed to an erosion mechanism. In our case erosion led to a 10% reduction in matrix diameter with respect to initial value. Subsequently, maximum reduction of 16% and 13% for CA and CAS hydrogels, respectively, were obtained.

Besides, calcium alginate beads tend to shrink in the acidic environment. At low pH values (<4), the carboxylate groups of alginate are protonated and hence the electrostatic repulsion among these groups decreased, favoring shrinkage (Abd El-Ghaffar, Hashem,

Table 2
Kinectic parameters obtained from Ritger and Peppas model.

Hydrogel formulation	$k \times 10^2$	n	R^2_{cor}
CA	0.25	0.17	0.96
CAS	0.17	0.23	0.96

CA: calcium alginate beads; CAS: calcium alginate-starch beads.

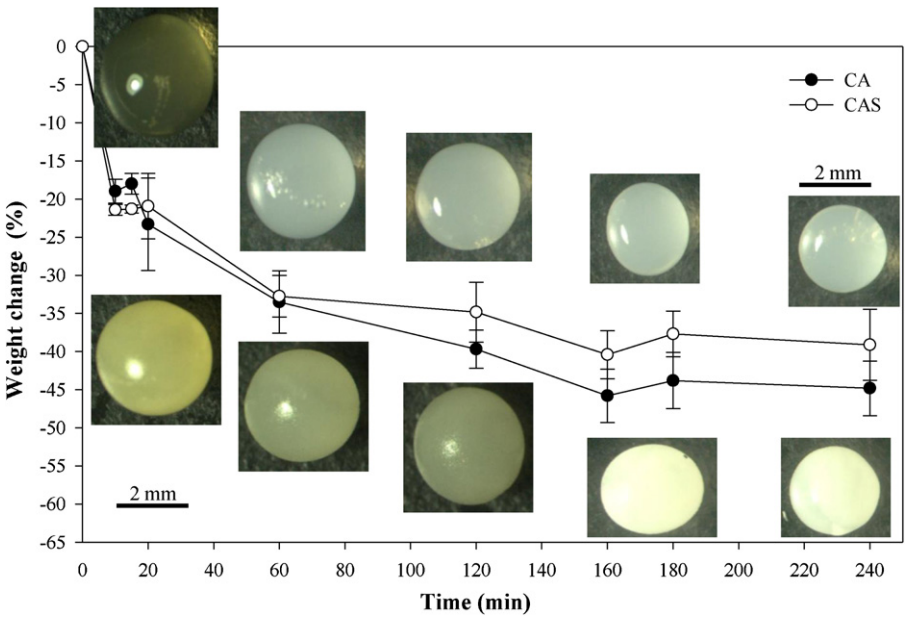

Fig. 7. Weight change kinetics of hydrogels beads in simulated gastric fluid. CA: calcium alginate beads; CAS: calcium alginate-starch beads.

El-Awady, & Rabie, 2012; Pasparakis & Bouropoulos, 2006). Thus in our work, the shrinkage of calcium alginate gel, could have partially contributed to the decrease of average diameter in CA and CAS hydrogels.

As was observed for size reduction, Fig. 7 also shows a mass loss. The more significant weight decrease was observed in the first 10 min of assay, with a mass loss of about 20% for both systems. Then, weight decrease continued up to 160 min, reaching approximately 40% reduction. After this time no significant mass change was detected. Thus, the relationship between the diameter reduction and the mass loss demonstrated that other mechanism besides diffusion is contributing to the overall polyphenol release.

Based on the above comments, the kinetic release data of the hydrogels was fitted to another empirical model were erosion is also taken into account, and is valid for a percentage release $\leq 70\%$ (Kopcha, Lordi, & Tojo, 1991):

$$M = At^{1/2} + Bt \quad (7)$$

In the above equation, M is the percentage of polyphenols released at time t, while A and B are the diffusion and erosion terms, respectively. According to this equation, if diffusion to erosion ratio is $A/B = 1$, the release mechanism includes both diffusion and erosion equally; if $A/B > 1$, then diffusion prevails, while for $A/B < 1$, erosion predominates.

As can be seen in Fig. 8 parameters A and B were determined at different time intervals, CA hydrogels showed predominance of erosion relative to diffusion, in the first 5 min of assay. Then, the diffusion term increased (A) and erosion term (B) decreased rapidly among 5–10 min. At major times the contribution of erosion phenomenon was insignificant because B values were below 0, so a diffusion mechanism governed the kinetics ($A \gg B$) (Fig. 8). In the case of CAS hydrogels a similar behavior was observed. But, in the first 5 min, although there is a contribution of erosion mechanism it is very low compared to diffusive contribution ($B \ll A$). In these samples, diffusion predominated during all the release kinetics and the contribution of erosion was negligible ($B < 0$). As mentioned earlier, the location of starch granules into the gel network diminished matrix erosion.

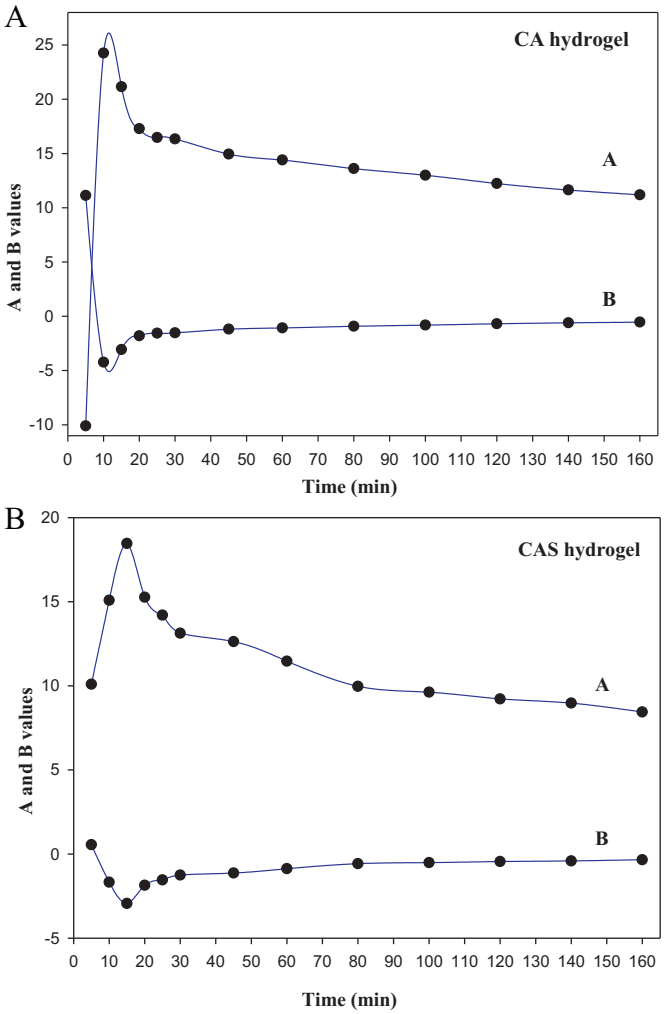

Fig. 8. Diffusional (A) and erosional (B) terms of Kopcha model versus time for CA and CAS hydrogels in SGF pH 2.

4. Discussions

Antioxidant activity of yerba mate extract has been proved by several authors through different in vitro and in vivo assays. This extract has shown a high ability as free radical scavenger, to reduce metal ions and to inhibit the lipid oxidation reaction (Bravo, Goya, & Lecumberri, 2007; Filip, Lotito, Ferraro, & Fraga, 2000). The encapsulation technique was used as strategy to improve handling of the active compounds by converting the liquid extract into a solid. In this work, the encapsulation of YM extract in calcium alginate hydrogels (with and without starch filler) did not affect the antioxidant power of the extract as denoted by the antioxidant capacity towards DPPH•. This fact was verified by FT-IR analysis, which proved the presence of active compounds in alginate matrix. Similar observation were found by Stojanovic et al. (2012) for calcium alginate beads loaded with thyme aqueous extract. Thyme polyphenolic compounds appeared to be chemically stable and antioxidant activity was preserved upon immobilization.

The addition of native starch improved encapsulation efficiency. As mentioned earlier, one of the limitations of calcium alginate hydrogels is the active compound loss during bead preparation (George & Abraham, 2006; Gouin, 2004). This disadvantage was partially overcome with the presence of filler material. Starch granules filled the voids and increased the tortuosity of the matrix, modulating the diffusion of active compound into the gelling bath. Besides, CAS hydrogels showed a modulating effect in the kinetics release of polyphenols observed in simulated gastric fluid.

During SGF immersion, the high release of polyphenols in the first minutes of the kinetics could be the sum of the presence of YM extract on the surface of the beads and the simultaneous erosion which causes around 20% of mass and 10% size reduction for both encapsulating systems. However, for this period, the corresponding released amount of yerba mate polyphenols was 32% for CA and 25% for CAS.

The commonly used Ritger and Peppas model fitted release data satisfactorily, even though the contribution of erosion mechanism was not included in this model. However, since our results suggested that a combination of diffusion and erosion mechanism could contribute to the overall release, Kopcha model was analyzed. This model showed that erosion had a reduced participation on polyphenol release compared to diffusion. The use of Kopcha model helped understand the difference between CA and CAS hydrogel behavior. The model demonstrated that erosion only had a significant contribution during the kinetic beginning for CA beads, because erosion in CAS beads was almost negligible in terms of polyphenol release. Probably starch granule presence contributed to a better distribution of the extract within the beads, decreasing the consequences of surface erosion and leading to a modulating effect.

Both encapsulating systems allowed totally recovery of entrapped polyphenols. Although CA and CAS beads showed different release rates, encapsulated compounds were totally recovered after the consecutive passage by the simulated gastric and intestinal fluids.

5. Conclusions

In general, starch addition did not affect antioxidant capacity of yerba mate extract, hydrogels morphology or their physical characteristics. The release of polyphenols in acidic media for both encapsulation systems was governed mainly by diffusion; only for calcium alginate beads erosion mechanism showed a slight contribution.

Incorporation of the starch filler to alginate matrix increased the entrapment capacity of yerba mate polyphenols, modulated the antioxidants release rate and diminished the contribution of matrix erosion to the whole release mechanism. Hydrogels reinforced with corn starch granules improved the classic calcium alginate system leading to a promising strategy to protect and deliver yerba mate antioxidants into food products.

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