Carbohydrate Polymers 99 (2014) 150–157

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Release of yerba mate antioxidants from corn starch–alginate capsules as affected by structure

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ARTICLE INFO

Article history:
Received 3 May 2013
Received in revised form 11 July 2013
Accepted 13 August 2013
Available online 22 August 2013

Keywords:
Natural antioxidants
Porosity
Encapsulation
Calcium alginate
Starch
Controlled release

ABSTRACT

Encapsulation of yerba mate (*Ilex paraguariensis*) extract in a proper matrix enhances the possible applications of this natural antioxidant in food systems. To start, calcium alginate capsules were used as carriers of yerba mate extract and a filler material (corn starch at 2%) was added to the alginate matrix to improve the structural properties and to modulate the release of the active compounds. Next, kinetics and swelling mechanisms involved in the release of yerba mate polyphenols in simulated digestive fluids were analyzed. A lower rate of release was obtained with calcium alginate–starch capsules as compared to control ones, which was attributed to the lower porosity of filled capsules. The release profiles of both systems were satisfactorily fitted with semi-empirical models, which indicated that a combined mechanism of polymer-chain relaxation and diffusion was taking place.

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1. Introduction

Yerba mate is listed as GRAS (generally recognized as safe) and constitutes a promising alternative for low-cost natural antioxidants to extend shelf-life of foods or act as a functional additive. Moreover, these extracts become an actual choice of synthetic additives since the latest alternatives have been associated with the current generation of adverse health effects (Moure et al., 2001). However, this substitution is still under study because natural antioxidants have limited stability under environmental conditions and some of them have an unpleasant flavor (Kosaraju, Labbett, Emin, Konczak, & Lundin, 2008).

The polyphenols are abundant compounds in yerba mate extract and some of its pharmacological properties have been related mainly to the high content of chlorogenic acid and its derivatives (Jaiswal, Sovdat, Vivan, & Kuhnert, 2010; Marques & Farah, 2009). Recent studies have demonstrated in vitro and in vivo antioxidant activities, and hepatoprotective, choleretic, diuretic, hypocholesterolemic, antirheumatic, antitrombotic, antiinflammatory, antiobesity or antiageing properties (Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2011; Dugo et al., 2009). In addition, research literature provides examples of yerba mate extracts used as ingredients to prevent deterioration of meats and meat products (De Campos et al., 2007; Racanicci, Menten, Alencar, Buissa, & Skibsted, 2011) and as a natural source of caffeine in energy drinks (Ferrari Pereira Lima, De Dea Lindner, Soccol, Parada, & Soccol, 2012; Heckman, Sherry, & De Mejia, 2010). However, only a few applications have reached the market (http://www.lifetreeorganics.com.au/Mat–Products.html).

The process of encapsulation has proved to be a powerful technique based on the envelopment effect of a polymeric matrix, creating a microenvironment in the capsule that is able to control the interactions between the internal and external environments and protect the encapsulated compound from factors that may cause deterioration such as temperatures, light, pH, moisture, and oxygen (Borgogna, Bellich, Zorzin, Lapasin, & Cesàro, 2010). Sodium alginate is soluble in water and can form gel beads by dropping an aqueous solution into a divalent or polyvalent cation solution (e.g. Ca^{2+}, Zn^{2+}) to encapsulate active compounds (Draget, Steinsvåg, Onsøyen, & Smidsrød, 1998; George & Abraham, 2006). However, due to the losses of active compounds occurred during preparation of alginate beads, the ionic gelation technique is still under optimization so that it can be applied on an industrial scale. Moreover, the high-diffusion rates of active materials through alginate macroporous matrix limit their application as controlled release systems (Hua, Ma, Li, Yang, & Wang, 2010; Malakar, Nayak, & Das, 2013). To face the high permeability of calcium alginate capsules, several methods like absorption of active compounds, the addition of others polymer coatings or filler materials, have been proposed (Chan, Yim, Phan, Mansa, & Ravindra, 2010; Gal & Nussinovitch, 2007; Rassis, Saguy, & Nussinovitch,

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http://dx.doi.org/10.1016/j.carbpol.2013.08.026

2002). In this sense, the natural fillers like starch granules offer certain specific advantages over inorganic fillers or synthetic polymers, such as easy availability, biocompatibility, biodegradability, non-toxicity, environmental friendliness and low price (George & Abraham, 2006; Nayak, Das, & Maji, 2012). Some authors have reported the encapsulation of natural antioxidant extracts (Chan et al., 2010; Lee, Kim, Chung, & Lee, 2009; Stojanovic et al., 2012). However, the interaction between the active compound and the encapsulating matrix, as well as the release kinetics should have a specific approach due to the composition diversity of natural extracts.

Controlled release may be defined as a method by which one or more compounds are made available at a time, under particular conditions (pH, enzymes, temperature, etc.), at a specific rate (Barbosa-Cánovas, Ortega-Rivas, Juliano, & Yan, 2005). The most important mechanisms that regulate the release rate of an active compound are diffusion, swelling, biodegradation/erosion and osmotic pressure (Pothakamury & Barbosa-Cánovas, 1995). The relevance of each will depend largely on the composition of the polymeric matrix and the surrounding fluid, whereby the active agent can be released to the medium by one or several mechanisms operating simultaneously (Barba, d'Amore, Chirico, Lamberti, & Titomanlio, 2009). Mathematical modeling plays an important role in facilitating polymeric network design by identifying key parameters and molecule release mechanisms (Mastromatteo, Mastromatteo, Conte, & Del Nobile, 2010).

The aim of this work was to study the kinetics and release mechanism of yerba mate polyphenols entrapped in dried calcium alginate matrixes in digestive simulated media. The effect of starch filler in the structure of the alginate matrix was evaluated as well.

2. Materials and methods

2.1. Preparation of capsules

Antioxidant extracts were obtained from a commercial yerba mate (Las Marías, Corrientes, Argentina), as described by Deladino, Anbinder, Navarro, and Martino (2008). Briefly, 3 g of yerba mate 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 and kept in dark flasks until used. Total polyphenol content was determined by the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Chlorogenic acid (Fluka, USA) was used as standard.

Alginate capsules with and without starch filler were prepared by the ionic gelation technique as described in a previous work (López Córdoba, Deladino, & Martino, 2013). For control capsules (CA) preparation, 2 g of sodium alginate (Sigma–Aldrich, USA) were dissolved in 100 mL of the yerba mate extract. The filled capsules (CAS) were obtained adding 2 g of commercial corn starch (Molinos Río de la Plata, Argentina) into 100 mL of the yerba mate-alginate solution described previously. Once homogenized and degassed by ultrasound, the solutions were forced with a peristaltic pump (Gilson Minipuls 3, France) into a syringe (diameter: 1 mm) to drop into a calcium chloride solution (0.05 mol/L). The capsules were maintained in the gelling bath to harden for 15 min. Then, they were filtered and washed with a buffer solution (acetic–acetate, pH 5.5).

Next, capsules were dried in a convection oven (SanJor, Argentina) at 65 °C until reaching constant weight and a water activity (a_w) of about of 0.53. After dehydration, the capsules were maintained in desiccators with silica gel until use.

2.2. Characterization of capsules

2.2.1. Antioxidant activity of encapsulated yerba mate extract

Antioxidant activity 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). A known amount of capsules was disintegrated by crushing and blended with an appropriate volume of distilled water. An equivalent amount of wet capsules was also assayed. The capsules were placed in an orbital shaker (Orbit Environ Shaker, Lab Instruments, USA) for 20 h, at 37 °C and 180 rpm. An aliquot of 100 μL of supernatant was removed and mixed with 3.9 mL of DPPH• ethanol solution (25 mg DPPH•/L). Absorbance was determined at 517 nm until the reaction reached a plateau. Antioxidant activity was expressed as the inhibition percentage (%*I*) of the free radical DPPH, calculated with the following equation:

$$\%I = \left(\frac{Abs_b - Abs_s}{Abs_b}\right) \times 100 \quad (1)$$

where Abs_b is the absorbance of control reaction (without the sample) and Abs_s is the absorbance of the sample.

A relationship between antioxidant capacity and total polyphenols content was determined for unencapsulated yerba mate extract at different concentrations.

2.2.2. Scanning electronic microscopy (SEM)

Analysis was performed using a FEI, Quanta 200 microscope (Netherlands). Capsules 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. Cross-cuts were obtained by cryo-fracture, immersing the capsules in liquid nitrogen.

2.2.3. Mercury intrusion porosimetry

The pore size of the capsules was estimated by mercury intrusion porosimetry using Pascal 440 (Thermo Fisher Scientific, USA) equipment, which allows determining pore size diameter until 0.2 nm, with pressures ranging from 0 to 400 MPa. The experimental data treatment was based on the Washburn equation (Washburn, 1921):

$$r = 2\sigma \cos\alpha / p \quad (2)$$

where p is the applied pressure, r is the radius of the pore, σ is the surface tension of Hg and α is the contact angle between mercury and the studied sample.

2.2.4. Determination of glass transition temperature by differential scanning calorimetry (DSC)

Glass transition temperature (T_g) was determined using DSC Q100 (TA Instruments, USA) equipment, which was calibrated with an Indium standard. Samples of 3–5 mg were placed in aluminum pans hermetically sealed and an empty pan was used as a reference. The samples were heated from 25 °C to 300 °C at a heating rate of 10 °C/min. Assays were performed in duplicate.

2.3. Release kinetics of yerba mate polyphenols in simulated digestive fluids

A modified method from "Delayed dosage forms: Method B" of US Pharmacopeia was performed (USP 34, 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 capsules with 25 mL of SGF was placed in an orbital shaker at 37 °C and 180 rpm.

Table 1
Effect of drying process on the antioxidant activity.

Capsule type	Condition	Antioxidant capacity (%*I*)
CA	Wet	74.15
	Dried	69.18
CAS	Wet	77.02
	Dried	69.69

CA: calcium alginate capsules; CAS: calcium alginate–starch capsules.

Aliquots of 200 μL of supernatant were removed at different times for 3 h. After this period, capsules were filtered and placed in another Erlenmeyer with 25 mL of SIF for 2 h.

The percentage of released polyphenols was calculated as follows:

$$\text{Released polyphenols (\%)}: \quad \frac{M_t}{M_\infty} \times 100 \tag{3}$$

where M_t is the mass of polyphenols quantified at each time t and M_∞ is the mass of yerba mate polyphenols loaded in the capsules. The value of M_∞ was determined adding a known amount of capsules to a sodium citrate solution (5 g/100 mL). Capsules were placed in an orbital shaker at 37 °C and 180 rpm for 3 h. The polyphenol mass was quantified by the Folin–Ciocalteau method mentioned above.

2.4. Swelling studies

Several flasks containing the same amount of capsules and volume of SGF as described in Section 2.3 were placed in the orbital shaker at 37 °C and 180 rpm. The swollen capsules were taken out at different times and the wet weight was determined immediately after absorbing the excess water from the surface with Whatman Grade 1 filter paper. The swelling ratio (%*Sw*) of the capsules was calculated with the following equation:

$$\%Sw = \left(\frac{W_t - W_0}{W_0}\right) \times 100 \tag{4}$$

where W_t and W_0 are the weight of the capsules in the swollen state at time t and the initial weight of the dried capsules respectively. All the experiments were performed in triplicate.

Also, morphological changes during the immersion in SGF were photographically monitored using a stereomicroscope Leica MZ 10F with camera Leica DFC 490 (Leica, Germany).

2.5. Statistical analysis

Analysis of variance (ANOVA) and mean comparisons were carried out using the software SYSTAT INC. (Evenston, USA). Release data were fitted to non-linear models using the DDSolver software (Zhang et al., 2010). Unless indicated, a level of 95% of confidence ($\alpha = 0.05$) was used.

3. Results and discussion

3.1. Total polyphenols content and antioxidant activity of the capsules

The polyphenols content for control capsules (CA) was 17.3 and for starch filled capsules (CAS) was 19.5 mg of chlorogenic acid/g dry capsules. A satisfactory linear correlation between antioxidant capacity and total polyphenols content was obtained ($R^2 = 0.99$) for free extract. This regression allowed estimating the expected antioxidant capacity for the yerba mate extract within capsules and, to compare these values with the measured antioxidant activity.

Table 1 shows the effect of the drying process on the antioxidant activity of the encapsulated extract. The type of encapsulation system (CA and CAS) and drying were not significant factors ($p > 0.05$). Besides, when comparing the measured antioxidant activity with the expected values calculated from the regression (polyphenols content vs. DPPH• inhibition), non-significant differences were found. The unencapsulated extract showed expected values for %*I* of 71.33% for CA and 77.17% for CAS. Thus, the encapsulation method used did not modify the antioxidant activity, regardless of the addition of starch filler. This fact is in agreement with the FT-IR analysis presented in a previous work where no interactions were detected between yerba mate polyphenols and the encapsulating matrix (López Córdoba et al., 2013).

3.2. SEM analysis

The calcium alginate capsules showed some surface cracks and an empty inner core (Fig. 1a and b). In calcium alginate–starch capsules the filler occupied mainly the inner core of the alginate matrix (Fig. 1e). Some starch granules were embedded into the surface, as well (Fig. 1d). Granule appearance could indicate that the temperature of the drying process was not high enough to produce starch gelatinization.

Before the drying process, the shapes of all the capsules were spherical regardless of the presence of starch. After drying, the shape of the calcium alginate capsules was irregular (Fig. 1c), while starch-filled capsules almost maintained the initial morphology (Fig. 1f). This suggests that the filler was able to act as a structural support to control the shrinkage and to maintain the shape of the beads after drying.

3.3. Mercury intrusion porosimetry

Mercury intrusion–extrusion curves for calcium alginate and calcium alginate–starch capsules can be observed in Fig. 2. During mercury intrusion, as the applied pressure increases, it causes the next smaller group of pores to be filled with a simultaneous increased in the total volume of mercury. Afterwards, the extrusion curve is obtained by depressurization of the sample. Two general phenomena are revealed, namely hysteresis between intrusion and extrusion, and the entrapment of mercury when the pressure is reduced to atmospheric pressure (Day et al., 1991; Karathanos & Saravacos, 1993). The difference in shape between the intrusion–extrusion curves of the two types of capsules indicated an effect of starch presence on capsule structure. The topology and pore geometry of the calcium alginate matrix should have been modified by starch granules, besides the pore size distribution.

Day et al. (1994) analyzed different mercury intrusion–extrusion curves of several systems to better understand entrapment mechanism and hysteresis. Experimental data on these well characterized systems allowed these authors to propose five classes of behavior. Calcium alginate capsules (Fig. 2), were characterized as Type I, described by a steep intrusion curve and an extrusion curve that led to a narrow parallel hysteresis loop with a small degree of entrapment. This behavior represents a narrow unimodal system. Calcium alginate–starch capsules also showed a steep intrusion curve, but lower than for capsules without filler, and an almost horizontal extrusion, which according to Day et al. (1994) corresponds to Type III, which represents a complex system containing either a broad distribution of pores or very narrow entrances into larger cavities.

As proposed by Dullien and Batra (1970), the behavior of the intrusion–extrusion curves may be used to indicate the pore shape of the material, as well. In general, the pore shape can be divided into three different types: cylindrical, conical and ink-bottle. The rapid penetration part of the intrusion curve for alginate capsules

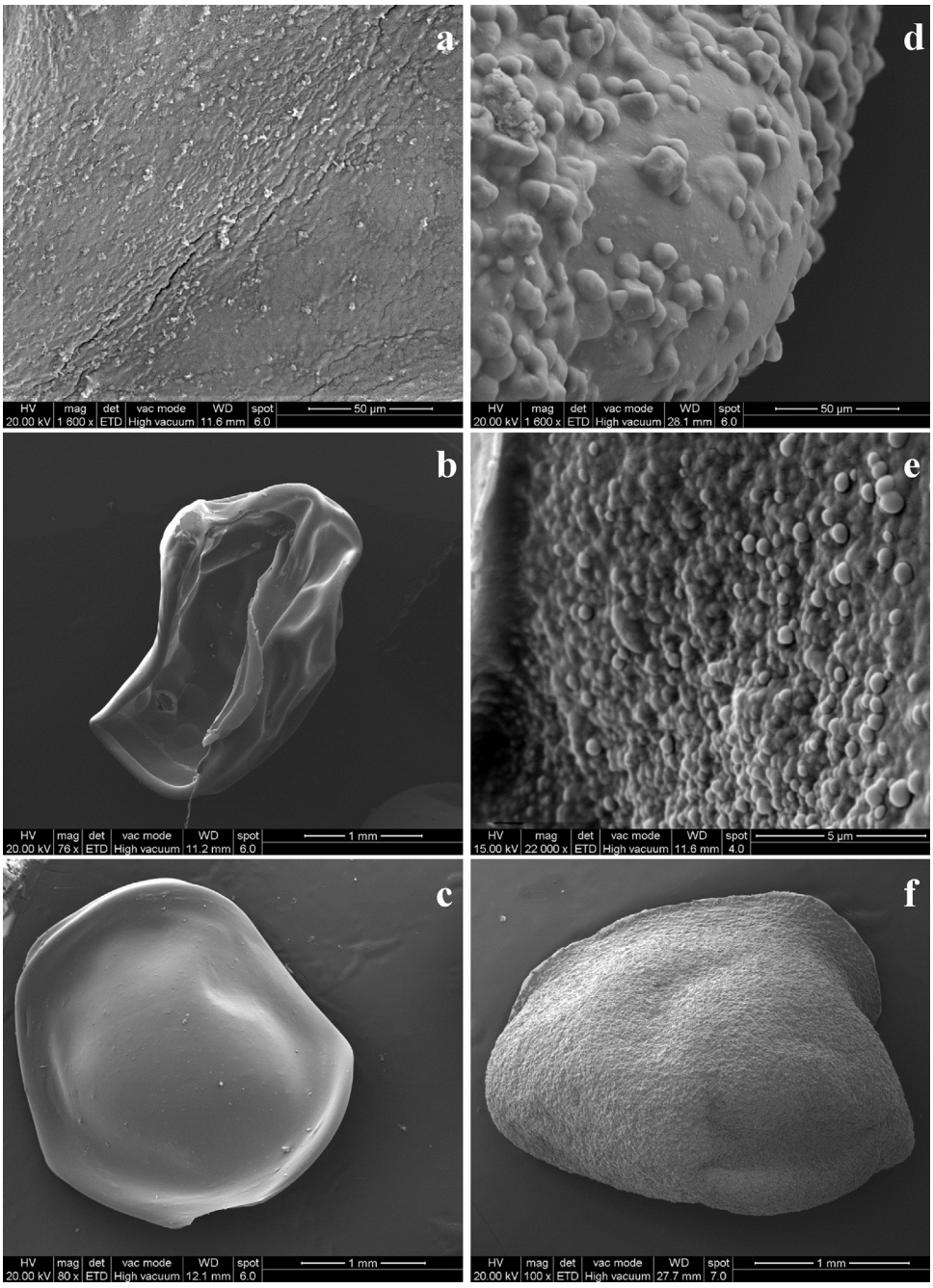

Fig. 1. SEM micrographs of calcium alginate capsules (a–c) and calcium alginate–starch capsules (d–f).

(CA) suggests the presence of cylindrical pores. The extrusion part has a similar shape as the intrusion one. A negligible amount of intruded mercury remained in the pores.

In the case of calcium alginate–starch capsules (CAS), the intrusion–extrusion curve obtained are characteristic of an ink-bottle shape, which can be described as a larger central cavity linked to a surface by smaller-diameter capillaries. Similar curves were observed by Chan et al. (2011) working with cells encapsulated in lyophilized alginate–starch beads.

After applying the Washburn equation, pore size distributions for CA and CAS capsules were calculated (Fig. 3). Pore sizes between 1 and 10,000 nm were obtained with an average diameter of 100 nm for both types of capsules. These results agree with other reports for similar encapsulation systems (George & Abraham, 2006; Klein, Stock, & Vorlop, 1983). However, the pore size distribution of

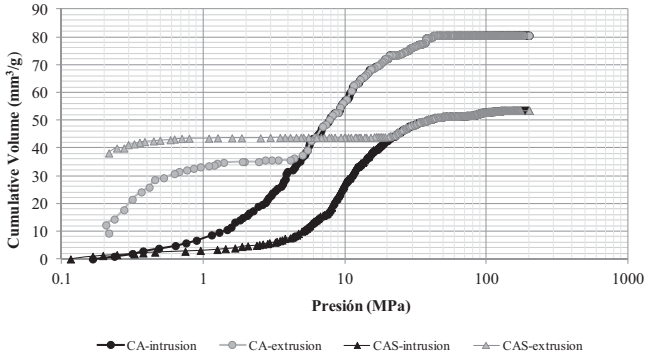

Fig. 2. Mercury intrusion–extrusion curves of calcium alginate (CA) and calcium alginate–starch (CAS) capsules.

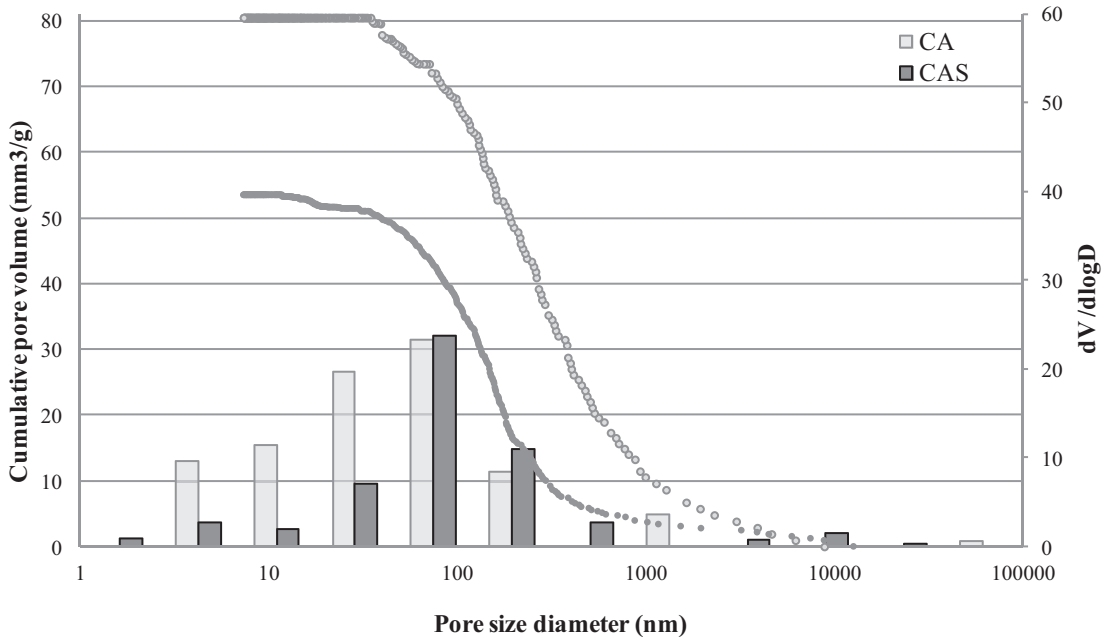

Fig. 3. Pore size distribution for the calcium alginate (CA) and calcium alginate–starch (CAS) capsules. Main axis: cumulative pore volume (Line graph), secondary axis: $dV/d\log D$ (Bar graph).

both types of capsules was different with CA capsules showing a higher total pore volume than CAS ones (Fig. 3). According to IUPAC, pores can be classified as follows: macropores, with diameters larger than 50 nm; mesopores, with diameters between 2 and 50 nm; and micropores, with diameters smaller than 2 nm (Sujka & Jamroz, 2007). Starch filler occupied the void spaces of the matrix decreasing the number of pores mainly between 5 and 50 nm, which means that the mesopores of the calcium alginate matrix were reduced (Fig. 3).

3.4. Glass transition temperature

Glass transition temperatures were 55.8 °C for calcium alginate capsules and 55.9 °C for capsules with starch filler. T_g values around 120 °C have been reported for calcium alginate gels in the literature (Naidu, Sairam, Raju, & Aminabhavi, 2005; Nakamura, Nishimura, Hatakeyama, & Hatakeyama, 1995). In the present work, the low T_g values were attributed to a plasticization effect of the low molecular weight compounds present in the capsules, like polyphenols of yerba mate and the low amount of water remaining within the matrix ($a_w = 0.53$). Besides, T_g values around 50 °C had been previously reported by this working group when encapsulating a freeze dried yerba mate extract in calcium alginate–chitosan systems (Anbinder, Deladino, Navarro, Amalvy, & Martino, 2011). These values, higher than the ambient temperature, are indicative of good stability. The diffusion of oxygen, which alters the stability of bioactive compounds and antioxidant, is decreased in the glassy state (Kosaraju, D'Ath, & Lawrence, 2006). However, the matrix could become rubbery accelerating polyphenol release by diffusion with an increase of ambient temperature or humidity (Gabbott, 2008).

3.5. Release kinetics of yerba mate antioxidants in simulated digestive fluids

The release kinetics of yerba mate polyphenols in gastric and intestinal simulated fluids was studied. A high percentage of recovery of yerba mate polyphenols was obtained in acidic medium at 3 h of assay, around 75% and 91% for CAS and CA capsules, respectively (Fig. 4). The remaining content of encapsulated polyphenols was fully released, after the total disintegration of the capsules during immersion in simulated intestinal fluid. This demonstrated that the encapsulated yerba mate polyphenols were not affected under gastric-intestinal conditions. Also, previous studies indicated that even the addition of digestive enzymes did not modify polyphenols content (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007; Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Besides, the activity toward DPPH radical of the released extract during passage through acidic medium was monitored. An increase in the antioxidant activity with release time was observed, in agreement with the amount of total polyphenols detected. Non-significant differences were found between the measured antioxidant activity with the expected values calculated from the regression (polyphenols content vs. DPPH• inhibition).

Significant differences in release rate of yerba mate polyphenols ($p < 0.05$) were detected between both formulations. The first zone of the kinetic curve was characterized by a fast polyphenol release, liberating almost 50% in the first 5 min for CA capsules

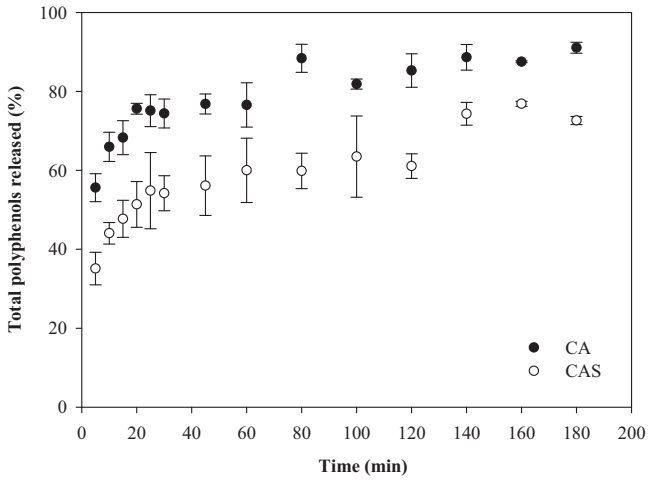

Fig. 4. Release kinetics of yerba mate antioxidants in simulated gastric fluid. CA: calcium alginate capsules; CAS: calcium alginate–starch capsules.

Table 2
Kinetic parameters obtained from the semi-empirical models.

Capsule type	Kinetic parameters			R^2_{cor}
	k_1			
First order kinetics				
CA	0.18			0.95
CAS	0.15			0.95
	k	n		
Power law model				
CA		not determined*		
CAS	30.46	0.16		0.97
	k_d	k_r	m	
Diffusion-relaxation model				
CA	45.74	0.60	0.18	0.98
CAS	23.16	4.91	0.18	0.96

*The relation M_t/M_∞ was >0.6 at first minutes. CA: calcium alginate capsules; CAS: calcium alginate–starch capsules.

and at 25 min for CAS capsules. In the second zone (30–180 min), a gradual release was observed. The effect of starch addition was a modified polyphenols release. This was attributed to the lower porosity of the alginate–starch matrix that with a torturous path makes polyphenol release more difficult. The rapid migration of polyphenol in the early stages of assay could be assigned to the drying process. According to Huang and Brazel (2001), active compound migration may occur during drying and storage. This process could lead to a heterogeneous distribution of the active compound in the matrix and provoke a fast release at initial times.

The release mechanism of yerba mate antioxidants was investigated using the empirical models (Peppas & Sahlin, 1989; Ritger & Peppas, 1987): first order (Eq. (5)), power law (Eq. (6)) and diffusion–relaxation model (Eq. (7)):

$$\frac{M_t}{M_\infty} = e^{-k_1 t} \tag{5}$$

where (M_t/M_∞) represents the fraction of mass released at time t (M_t) with respect to the maximum mass of polyphenols (M_∞) that would be released at time $t = \infty$ in all cases and k_1 is the kinetic constant:

$$\frac{M_t}{M_\infty} = kt^n \tag{6}$$

where k is a constant related to structural and geometric characteristics of the capsules and active compound and n is the transport exponent indicating the type of release mechanism involved. Eq. (6) is valid only for the first 60% of the release curve, which means that M_t/M_∞ is <0.6 (Ritger & Peppas, 1987):

$$\frac{M_t}{M_\infty} = k_d t^m + k_r t^{2m} \tag{7}$$

where k_d, k_r and m are constants. The first term represents the diffusional contribution and the second term represents the case-II relaxation contribution, taking into account the possibility of the two different mechanisms acting together. The goodness of fit of the models was evaluated using the corrected coefficient of correlation (R^2_{cor}) and the residual analysis. Other models like Zero-order, Higuchi, Hixson-Croswell and Bakers-Lonsdale have been tested, but model fitting to experimental data gave R^2_{cor} below 0.3.

Table 2 shows the kinetic parameters obtained from release curves for CA and CAS capsules. The first order model (Eq. (5)) showed a good fitting and the kinetic parameters were in agreement with the lower release rate of the alginate–starch capsules.

Calcium alginate capsules showed values of $M_t/M_\infty > 0.6$ in the first minutes. So, power law model (Eq. (6)) could only be applied to calcium alginate–starch capsules. The value of the diffusional exponent (n) was lower than 0.5, indicating that alginate–starch capsules exhibited a non-Fickian diffusion mechanism.

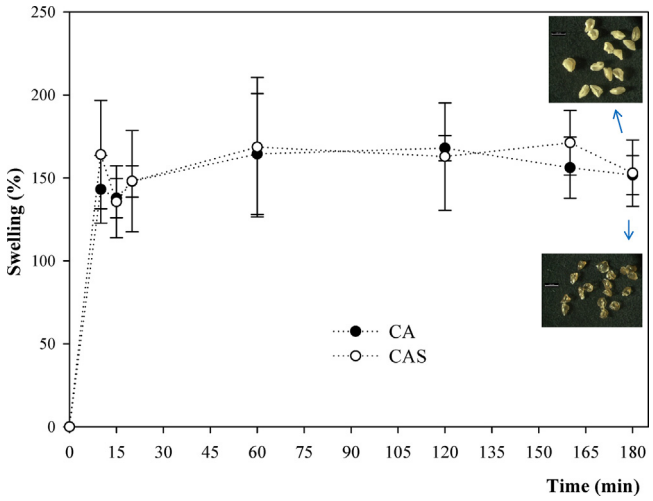

Fig. 5. Swelling behavior in simulated gastric fluid for the calcium alginate (CA) and calcium alginate–starch (CAS) capsules.

The best fit was obtained with the diffusion–relaxation model (Eq. (7)) for both systems. This model is related to an initial chain plasticization, which involves a reduction in the attractive forces between chains followed by swelling and relaxation mechanisms acting together, increasing the mobility and active compound migration (Llabot, Manzo, & Allemandi, 2004). The relationship between the two constants ($k_d > k_r$) suggests that the release mechanism is mainly diffusive, although the contribution of a relaxation mechanism was also observed (Table 2).

3.6. Swelling studies

As was described earlier, capsules are in the glassy state (T_g around 55 °C), where the mobility of molecules is prevented. In order to allow the diffusion of active compound within and from the matrix, a previous hydration, plasticization and/or relaxation of polymeric chains is necessary (Brazel & Peppas, 2000; Peppas & Khare, 1993). As shown in Fig. 5, both types of capsules swelled with immersion time in SGF. However, they did not recover the spherical shape observed before the drying process. As discussed above, the collapse during drying was lower in the starch capsules, which facilitates a better recovery of the structure.

The percentage of swelling (%*Sw*) was plotted against time to describe the swelling profile of the capsules in the simulated gastric conditions (Fig. 5). Positive values of %*Sw* and the upward trend refer to the overall swelling and weight gain of capsules with medium absorption. Non-significant differences were observed between the swelling profiles of the two studied systems. The maximum value of swelling (150%) was reached in the first period (0–20 min), then they reached a steady period in the capsule mass did not change significantly over time. These results correlate with those obtained in the release kinetics in which at 25 min more than 50% of the yerba mate polyphenols encapsulated in dry systems were released.

Swelling profiles of capsules fitted to a second order equation proposed by Schott (1992) (Eq. (8)):

$$\frac{dW}{dt} = b(W_\infty - W_t)^2 \tag{8}$$

where W_∞ is the maximum percentage of medium absorbed at equilibrium, W_t is the percentage of medium absorbed at time t

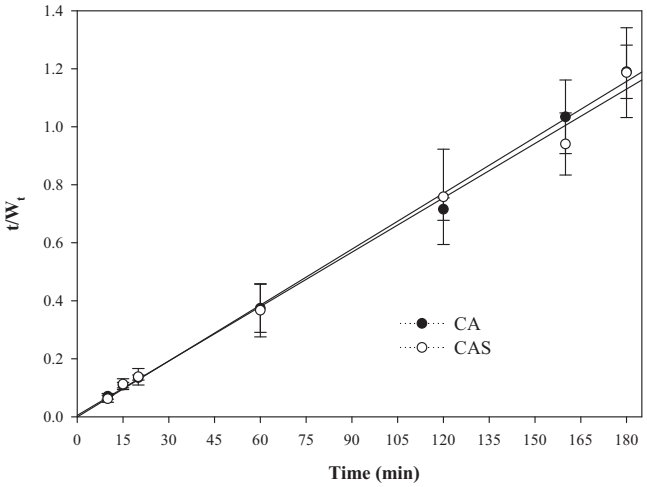

Fig. 6. Fitting of Schott equation (Eq. (9)) for calcium alginate (CA) and calcium alginate–starch (CAS) capsules.

Table 3
Parameters obtained from Schott equation.

Capsule type	Experimental W_∞	Estimated W_∞	$b \times 10^3$ (min^{-1})	R^2
CA	167.94	158.73	8.27	0.99
CAS	171.19	175.43	1.55	0.99

CA: calcium alginate capsules; CAS: calcium alginate–starch capsules.

and b is swelling rate constant. After integration between $[0, W_t]$ and $[0, t]$, Eq. (8) was transformed in:

$$\frac{t}{W_t} = \frac{1}{W_\infty} t + \frac{1}{bW_\infty^2} \quad (9)$$

Fig. 6 shows graph t/W_t vs. t, for CA and CAS capsules. The obtained curves showed a good fitting, with a high lineal correlation ($R^2 > 0.98$), indicating that the swelling profile of these systems obeys a second order kinetics. The model parameters, W_∞ and b, calculated by lineal regression are shown in Table 3. The calculated maximum percentages of absorbed medium (W_∞) from the model were similar to experimental data (Table 3). With respect to the estimated swelling rate (b), similar values were obtained for both systems, matching with the experimental observations where no differences were detected between both swelling profiles.

3.7. Discussion on the relationship between capsule microstructure and the release of active compounds

The release behavior of the active compounds highly depends on the polymer–active compound interaction, the physical state and microstructure of the capsules, and the surrounding medium. In a previous work, using equivalent encapsulation wet systems, the release mechanism in calcium alginate hydrogels was governed by erosion and diffusion acting together, whereas, only diffusion was relevant for starch loaded beads (López Córdoba et al., 2013). In the present study, working with dry systems, the polyphenols diffused while relaxation and swelling of the polymer matrix were taking place. In the dried state, starch filler played a relevant role retarding release rate.

The effect of different drying processes on morphology, size and release mechanism has been discussed by some authors, employing different methods or combination with other materials to retard release from calcium alginate capsules. Santagapita, Mazzobre, and Buera (2012) studied the effect of drying on morphology and size of alginate beads with trehalose and chitosan. They found that bead size was affected by drying even more than bead composition. In the mentioned work, freeze-drying allowed better maintenance of the diameter and the circularity of the beads, which showed smoother surface and smaller pores than those generated by vacuum or air drying. Stojanovic et al. (2012) found that the incorporation of inulin into the alginate matrix as a filler substance reduced its collapse and roundness distortion during the heating and freeze-drying processes. These authors found a modulating effect on the release kinetics of a thyme extract. Drying with microwave irradiation led to a release-retarding effect on alginate–chitosan beads by increasing the extent of polymer interaction, which depended on irradiation conditions (Wong, Chan, Kho, & Sia Heng, 2005; Wong, Chan, Kho, & Sia Heng, 2002).

As discussed earlier, the lower porosity found in alginate–starch capsules led to a retarding release effect. The starch would have generated more tortuous and rigid structure which makes diffusion of the active compound more difficult. In this sense, Chan et al. (2011) obtained X-ray images of lyophilized starch–alginate beads that revealed a 'spider-web' pattern in which the filler formed 'bridges' that connected the concentric layers. This tendency was attributed to the direction of the cross-linking process, which forms denser gel layers and, thus, less 'readily available space' at the surface compared to the inner core. The same effect was observed by other authors using inert filler materials such as bentonite, silicates, cellulose, among others (Gal & Nussinovitch, 2007; Nayak et al., 2012; Puttipipatkhachorn, Pongjanyakul, & Priprem, 2005; Rassis et al., 2002; Sultana et al., 2000).

In a previous work, chitosan coating was used with the aim of retarding polyphenols release (Deladino et al., 2008). Although other benefits were discovered, the release was not modulated, the capsules were too flat and porosity was still high. However, in the present work, the incorporation of a second material as filler effectively improved the structure of calcium alginate capsules. A modulating delivery system was created by filling alginate-matrix voids with natural granules.

4. Conclusion

Yerba mate extract was successfully encapsulated by the ionic gelation technique, while its high antioxidant activity was not modified even though corn starch was used as filler. Starch granules acted as a structural support improving the capsule morphology when compared with control alginate capsules. Both types of encapsulating systems were under the glassy state at ambient temperature.

The presence of starch granules within the matrix was responsible for the lower porosity and the change in pore size distribution, leading to a modulated release of yerba mate polyphenols in simulated gastric fluid. Both encapsulating systems released their extract content mainly by a diffusive mechanism. However, a relaxation/plasticization mechanism showed a contribution at the beginning of the release period.

The addition of corn starch granules into typical calcium alginate capsules was an easy, economical and functional approach to optimize the yerba mate antioxidant's protection and delivery into food products.

Acknowledgements

The authors would like to thank the Instituto Nacional de la Yerba Mate (INYM) for their support through the PRASY project, to the Argentinean National Research Council (CONICET) and to Lic. Edwin Barbosa and Eng. Christopher Young for English language supervision.

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