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Carrier systems for yerba mate extract (*Ilex paraguariensis*) to enrich instant soups. Release mechanisms under different pH conditions

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ABSTRACT

The high antioxidant properties of yerba mate (*Ilex paraguariensis*) extract make it suitable as a natural food additive. Encapsulation of herbal extracts is often necessary to enhance handling and product stability. Release strategies depend on the encapsulation system and the intended use. Calcium alginate capsules, with and without a chitosan layer, containing the yerba mate extract were analyzed, in wet and dried state. Polyphenol release was quantified in gastric (HCl solution, pH = 2) and intestinal (phosphate buffer pH = 7.4) simulated fluids. Most of the encapsulated polyphenols were released in the acidic medium. However, wet capsules with chitosan maintained a higher amount of the active compound before reaching the intestinal medium. Several models were tested to fit kinetic release data; wet and dry capsules showed different release mechanisms in the acidic solution. The dry capsules hydrated, while the wet ones eroded before releasing their content mainly by diffusion. Carrier systems with yerba mate extract were added to instant vegetable soups. Capsules addition increased polyphenols content without modifying the flowability of the soups. The sensory evaluation carried out by a non-trained panel showed no significant differences between enriched and commercial soups.

1. Introduction

Yerba mate (*Ilex paraguariensis*) is a tree native to South American countries (Argentina, Uruguay, Paraguay and Brazil) and the leaves, once processed, are consumed as an infusion. Several authors (Anesini, Ferraro, & Filip, 2006; Bracesco, Sanchez, Contreras, Menini, & Gugliucci, 2011; Heck & de Mejía, 2007; Schinella, Fantinelli, & Mosca, 2005) studied the antioxidant properties of yerba mate by using chemical models and *ex vivo* lipoprotein studies. These antioxidant characteristics were related to health benefits like antimutagenic and anti-glycation effects, vaso-dilating and lipid reduction properties, and weight reduction.

In a previous work, the microstructure of alginate capsules containing lyophilized yerba mate extract was characterized (Deladino, Anbinder, Navarro, & Martino, 2008). These capsules, with and without a chitosan layer, showed a high load of polyphenol compounds that were correlated with the antioxidant capacity. Thus, to gain insight into food applications, the release of yerba mate extracts from the capsules needs to be addressed. Kikuchi et al. (1999), working with dextran molecules ranging from 9400 to 145,000 Da, found that the release mechanism of alginate gel beads depended on molecular weight. Macromolecules release from alginate beads in low pH solutions is significantly reduced, thus leading to an advantage in the development of oral delivery systems (George & Abraham, 2006). The limitations of encapsulated systems using a polymer sensitive to pH could be solved by combining polymers of different characteristics to enhance individual release properties. For example, the carboxylic residues of alginate and the amino groups of chitosan interact ionically to form a polyelectrolyte complex (Anbinder, Deladino, Navarro, Amalvy, & Martino, 2011). This complex could reduce the porosity of wet alginate capsules and decrease the leakage of encapsulated materials (Huguet & Dellacherie, 1996; Sezer & Akbuga, 1999). Theoretically, chitosan solubility at the low pH of the gastric system could be diminished by the alginate network, which is insoluble under gastric condition. In a similar way, the alginate dissolution at high pH levels could be modulated by the presence of chitosan. Many authors have applied the alginate–chitosan complex properties to encapsulate and control the release of drugs and other active substances (Abreu, Bianchini, Forte, & Kist, 2008; Anal & Stevens, 2005; Bartkowiak & Hunkeler, 2000; Gåserød, Smidsrød & Skjåk-Bræk, 1998a; Gåserød, Jolliffe, Hampson, Dettmar & Skjåk-Bræk, 1998b; Kim, Chung, Shin, Yam, & Chung, 2008;

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Pasparakis & Bouropoulos, 2006). However, the combination of encapsulating materials and active compounds has specific characteristics that should be taken into account.

The ability to release the active compounds depends on the nature of the encapsulating polymers and the target medium. Temperature, solvents, pH variations and mechanical stress are the most used conditions to liberate the active compounds. Release mechanisms are commonly carried out under *in vitro* conditions, before addressing *in vivo* studies. Literature provides several models to simulate human digestion, with different degrees of complexity. Real conditions involve too many factors that complicate the modeling of release processes focused on polymer behavior. Therefore, simple systems involving different pH conditions are considered before any additional factors can be incorporated into the simulated gastrointestinal system. The US Pharmacopeia (USP 34, 2011) methods offer different *in vitro* dissolution or release tests. Stulzer et al. (2008), working on cross-linked malonylchitosan microspheres for controlled release of acyclovir, used pH = 1.2 and phosphate buffer pH = 7.4 as an *in vitro* gastrointestinal system. Sansone et al. (2011), when encapsulating flavonoid microparticles by spray-drying, also employed acidic media to emulate stomach pH conditions and phosphate buffers to imitate intestinal pH conditions. When studying medicinal plant extracts, Belščak-Cvitanović et al. (2011) used water as a solvent to release the antioxidants from the alginate–chitosan system. Santagapita, Mazzobre, and Buera (2012) while studying enzyme release from alginate beads employed sodium acetate buffer 50 mMol/L (pH 3.8, 4.5, or 5.5). A more complex system included enzymes like pepsin and pancreatin in pH solutions that match conditions found in the human body (Maltais, Remondetto, & Subirad, 2010). Bile salts were also employed to simulate the small intestine (Cilla et al., 2011; Wootton-Beard, Moran, & Ryan, 2011).

New trends in the food industry are focused on finding natural compounds to enrich foods, due to the change in population habits and the constant search for healthier nourishment. In this regard, an instant vegetable soup came up as an appropriate food to enrich with extra natural antioxidants from yerba mate.

The objectives of the present work were to model the release kinetics of yerba mate extracts from alginate–chitosan capsules in gastric and intestinal simulated fluids. Physico-chemical and sensory evaluations were carried out to test the possibility of adding encapsulated antioxidants to a commercial product.

2. Materials and methods

2.1. Capsules preparation

The active compound was a lyophilized yerba mate extract obtained as described in a previous work (Deladino et al., 2008). The encapsulating agents were 2 g/100 mL sodium alginate solution (Protanal, Norway) and 1 g/100 mL chitosan dissolved in 1 g/100 mL acetic acid (Sigma–Aldrich, USA).

The yerba mate extract (1 g/100 mL) was mixed with the sodium alginate solution. Once homogenized, the solution was forced with a peristaltic pump (Apema, PC25, Argentina) 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 through a Whatman #1 paper and washed with buffer solution (acetic-acetate, pH 5.5). Beads were allowed to stabilize in air for 15 min. Some calcium alginate capsules were immersed in a chitosan solution for 30 min. These beads will be referred as wet capsules. Part of the wet capsules were dried in a convection oven at 65 °C for 3 h, further on, they will be referred as dried capsules. Briefly, two types of capsules were prepared: Calcium Alginate capsules with yerba mate extract (AY) and Calcium alginate–chitosan capsules with yerba mate extract (ACHY).

2.2. Loading efficiency

Loading efficiency of capsules was calculated as described in a previous work (Deladino et al., 2008) by disintegrating the calcium alginate matrix with sodium citrate 5 g/100 mL and with different pH media. These solutions were selected to simulate the conditions of human digestion. Chlorhydric Acid (Anedra, Argentina) 0.1 mol/L was used to simulate gastric fluid. The pH of the solution was adjusted to pH = 2 with 1 mol equiv/L NaOH. Intestinal fluid was simulated with Sorensen's phosphate buffer pH = 7.4.

Assays with simulated fluids were performed with 40 wet or dry capsules. The number of capsules employed corresponded to 10 mg of encapsulated extract (theoretical extract mass). An erlenmeyer containing the beads immersed in 100 mL of pH = 2 solution was placed in an orbital shaker (Orbit Environ Shaker, Lab Instruments, USA) at 37 °C and 180 rpm for 3 h. After this period, capsules were filtered and placed in another erlenmeyer with 100 mL of the buffer solution for 3 h. To analyze the release effect of each medium, total polyphenol mass was quantified in both simulated fluids by the Folin–Ciocalteau method (Schlesier, Harwat, Böhm, & Bitsch, 2002). Two 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).

Loading efficiency was calculated with the following equation:

$$\text{Loading efficiency}(g/100g) = \frac{L}{L_0} * 100 \quad (1)$$

Where L is the amount of extract determined in the solution of sodium citrate or simulated fluids and L_0 is the amount of extract dissolved in the alginate solution, called theoretical load.

2.3. Active agent release

The release kinetics of yerba mate extract was determined, under the same conditions as described above, taking 200 μl samples from pH = 2 and pH = 7.4 solutions at different times for 3 h. The assay was adapted from "Delayed release dosage forms. Method B" of US Pharmacopeia (USP 34, 2011). Total polyphenol content was determined as described previously (2.2). Assays were performed at least four times. The percentage of released extract was calculated as follows:

$$\text{Released extract}(g/100g) : \frac{L_t}{L} * 100 \quad (2)$$

Where L_t is the mass of extract quantified at each time and L is the mass of extract loaded in the capsules determined experimentally (2.2).

2.4. Enrichment of instant soups with encapsulated yerba mate extracts

Dried capsules were added to commercial instant soups (Knorr, Unilever, Argentina). The product was a low fat powder containing pieces of dehydrated vegetables (water activity, $a_w = 0.255$). Soups and capsules were mixed in dried state in a proportion of 44 mg capsules/g of soup placed in caramel flasks tightly sealed with parafilm® and stored at 23 °C. This amount of capsules corresponded to a theoretical amount of 1.5 mg of lyophilized extract.

Also, 44 mg of capsules (without soup) were stored as controls. Total polyphenols content was determined. For comparison purposes, polyphenols content in soups were expressed as mg of lyophilized yerba mate extract/mL of prepared soup, either with or without capsules.

2.4.1. Flowability test

Flowability of commercial (CS) and enriched soups with AY and ACHY capsules (AYS and ACHYS) was determined. Recently prepared and stored samples were analyzed using a repose angle chamber (Solids handling study bench, CEN, Armfield, United Kingdom). This device consists of a rotatable acrylic transparent graduated flat cylinder, which is part of a solids handling study equipment set (Fig. 1). The cylinder was half-filled with the sample and the surface was leveled. The device was rotated until the particles began to slide, at this moment, a reading was taken. Then, the camera was rotated in the opposite direction until the particles slid and a second reading was made. The mean value of both readings corresponded to the repose angle of the sample. All measurements were performed six times at ambient temperature.

2.4.2. Sensory analysis

The analysis was carried out with untrained volunteers who claimed to consume this type of product at least twice a week. Panelists were not trained, but prior to sample evaluation, they received instruction regarding the evaluation procedure in both written and verbal form. Two independent tests were carried out, one for AYS and the other for ACHYS soups, each one against the commercial soup without capsules. The same group of people performed both tests in two consecutive days.

Samples were presented in 50 mL plastic thermal vessels, randomly coded with three digit numbers. Panelists were asked to prepare the soups themselves, as suggested on the commercial package where the serving size was 11 g soup/250 mL of boiling water. Each of the 30 panelists received a form sheet with the following instructions: "Taste samples from left to right. Two of the samples are identical. Determine which one is the odd sample. You may re-taste samples. If no difference is apparent, you must guess" (Radovich, Kleinhenz, Delwiche, & Liggett, 2004). Panelists were requested to rinse their mouths with bottled spring water between samples. Statistical analysis was performed counting the number of right answers (right identification of the different sample). Depending on the total number of answers, data obtained was compared with tabulated critical values at different confidence levels (ASTM, 1968; Ureña, D'Arrigo, & Girón, 1999). If the number of right answers was equal to or higher than the corresponding critical value, samples were considered significantly different.

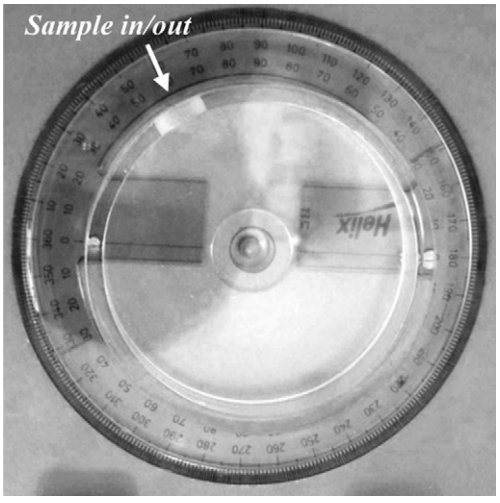

Fig. 1. Repose angle chamber employed to determine flowing properties.

2.5. Statistical analysis

Statistical data analysis was performed with the software SYSTAT INC. (Evanston, USA). Analysis of variance (ANOVA) and means comparisons were made. Also, non-lineal model application was used for fitting *in vitro* release data. Unless indicated, a level of 95% of confidence ($\alpha = 0.05$) was used.

3. Results and discussion

3.1. Loading efficiency

Active compound content was quantified as total polyphenols to facilitate experimental determinations, based on the good correlation between total polyphenol determination (Folin–Ciocalteau method) and the antiradical power measured by DPPH• technique (Deladino et al., 2008). Sodium citrate is a sequestering agent that disintegrates calcium–alginate capsules and has been previously used to calculate the loaded polyphenol content (Belščak-Cvitanović et al., 2011; Deladino et al., 2008). As can be seen in Table 1, polyphenol content quantified in simulated fluids (pH = 2 + pH = 7.4) was similar to that obtained with sodium citrate. These results indicate that the whole digestive system used in the present work released the total polyphenol content encapsulated in both systems (AY and ACHY).

The chitosan coated capsules had a lower amount of yerba mate extract when compared with the calcium alginate ones (Table 1). These capsules were obtained in a two stage process; calcium alginate beads formation (ionic gelation) and a later immersion in chitosan where a polyelectrolyte complex was formed. Some polyphenols were lost by diffusion in the chitosan solution and also a minor amount could have been retained taking part in the formation of the polyphenol–chitosan complex (Anbinder et al., 2011; Deladino et al., 2008; Popa, Aelenei, Popa, & Andrei, 2000).

To compare the contribution of each media to the total digestive system, the percentage of each digestive fluid was calculated with regard to the sum of pH = 2 and pH = 7.4 media, for both type of capsules. In this way, the polyphenol percentage quantified in pH = 7.4 solution could indicate the amount of encapsulated polyphenols reaching the intestine. For ACHY capsules, this value represented the 20% of the total amount of polyphenols released, while in capsules without the chitosan layer it was a 10% of the total (Table 1). This behavior could be attributed to the protecting barrier of chitosan that prevented polyphenol loses under gastric conditions. Thus, a higher amount of yerba mate polyphenols could reach the gut in the case of ACHY capsules.

3.2. Active agent release

As can be observed in Fig. 2, after passing through acidic medium, wet capsules preserved their integrity; whereas their

Table 1
Polyphenols released in different media.

Type of capsule (dried)	Sodium citrate	Acidic medium (pH = 2)	Basic medium (pH = 7.4)	Acid + basic media
AY	87.08* ± 7.29	81.69 ± 4.26	8.70 ± 0.31	90.39 ± 4.57
ACHY	48.48 ± 8.56	36.29 ± 3.88	9.52 ± 5.43	45.81 ± 9.31

AY = Alginate capsules with yerba mate extract, ACHY = Calcium alginate–chitosan capsules with yerba mate extract.
* g/100 g of loaded extract.

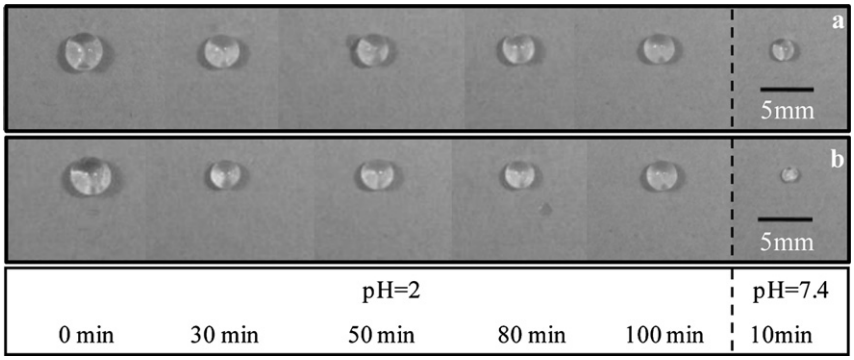

Fig. 2. Photographs of wet capsules showing the morphology after immersion in simulated fluids (pH = 2 and pH = 7.4). a) Calcium alginate capsules (AY), b) Chitosan coated alginate capsules (ACHY).

initial size was reduced, beads were hard enough to resist manipulation. The yerba's green characteristic color disappeared because of acidic conditions, while matrix material erosion was more evident in the pH = 7.4 medium. Both the AY and ACHY capsules lost their integrity after 20 min in this buffer solution.

In the case of capsules coated with chitosan (Fig. 2b), although they were disintegrated, some pieces of thin gel remained in the buffer solution. These slices could be assigned to the remaining complex of alginate–chitosan that resisted pH changes.

Fig. 3 shows that the dry capsules swelled in acidic medium, recovering almost their initial wet size after 30 min at this condition. Both types of dry capsules (AY and ACHY) were disintegrated after 10 min of immersion in pH = 7.4.

Fig. 4a–b shows kinetic release curves of yerba mate extract for both types of wet and dry capsules in simulated gastric fluid. The percentage of released extract was calculated based on the total polyphenol content determined after the pH treatment to simulate the conditions of human digestion. In wet capsule release (Fig. 4a), curves were parallel and ACHY capsules released a lower amount of polyphenols. However, the repeated measures ANOVA evidenced no effect of capsule type on the polyphenol release ($p > 0.05$).

While swelling, polyphenol release was taking place in dry capsules (Fig. 4b). Therefore, swelling process was not the limiting step in both encapsulation systems. Besides, as suggested by Huang and Brazel (2001) during drying, water moves to the surface promoting diffusion and migration of encapsulated compounds. Therefore, more soluble compounds may diffuse by convection with water, leading to an uneven compound distribution in the capsule with higher concentrations on the surface. This theory could explain the fast release in the first minutes of the kinetic curve of dry capsules (Fig. 4b).

The ANOVA assay with repeated measures indicated that the amount of polyphenols released by AY and ACHY dry capsules was not significantly different, along digestion time ($p > 0.05$).

3.3. Application of mathematical models

Several mathematical models for kinetic release can be found in literature (Arifin, Lee, & Wang, 2006; Popa et al., 2000; Pothakamury & Barbosa-Cánovas, 1995; Ritger & Peppas, 1987a, b). Some of these models were applied in the case of acid pH medium, where almost all the polyphenols were released. The semiempirical expression in Eq. (3) is the most commonly used model. In the present work, the exponential equation was applied as follows:

$$\frac{M_t}{M_\infty} = kt^n \tag{3}$$

where (M_t/M_∞) represents the fraction of mass released at time t (M_t) with respect to the maximum mass of polyphenols that would be released at time $t = \infty$. The maximum loaded mass of polyphenols (M_∞) was the mass quantified in pH = 2 solution, k is a constant related to structural and geometric characteristics of capsules and active compound and n is the transport exponent indicating the type of release mechanism involved.

Table 2 summarizes the range of n values associated to the transport mechanism determined in several works (Llabot, Manzo, & Allemandi, 2004; Pothakamury & Barbosa-Cánovas, 1995; Sumathi & Ray, 2002). Firstly, Ritger and Peppas (1987a) developed a simple method for the analysis of controlled release data for non-

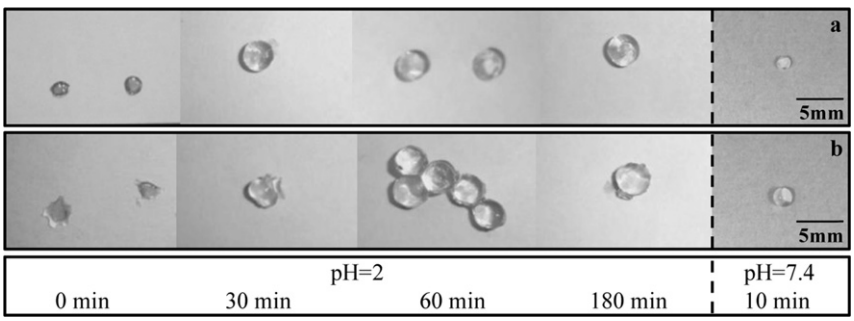

Fig. 3. Photographs showing dried capsules morphology after immersion in simulated fluids (pH = 2 and pH = 7.4). a) Calcium alginate capsules (AY), b) Chitosan coated alginate capsules (ACHY).

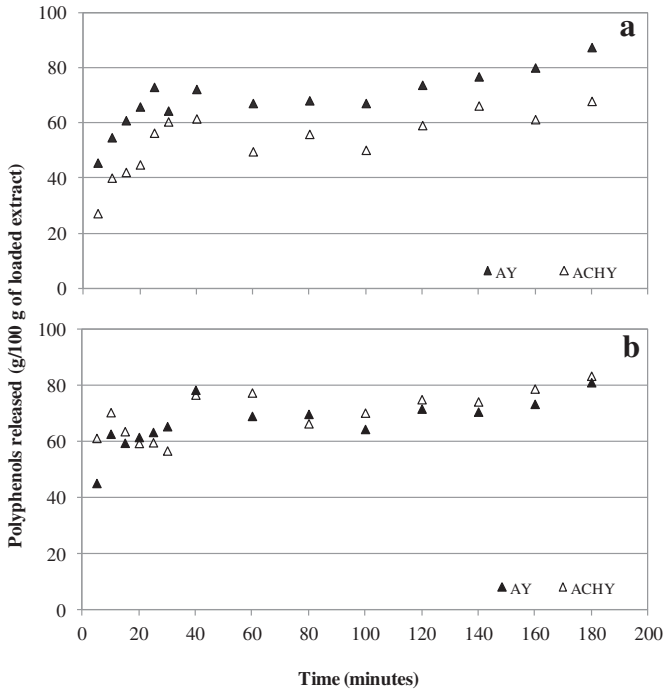

Fig. 4. Kinetic release curves of polyphenols in pH = 2 solution. (▲) alginate capsules with yerba mate extract (AY) and (Δ) calcium alginate–chitosan capsules with yerba mate extract (ACHY). a) Wet capsules, b) Dry capsules. Max. std. dev. = 15.

swellable polymeric systems. Later, the same authors (Ritger & Peppas, 1987b) considered that this model also could be applied if polymer swelling was moderated in water or in biological systems, establishing that it could not exceed 25% of the original system volume.

Eq. (3) is applicable for the first 60% of the release curve, which means that M_t/M_∞ is <0.6 (Costa & Sousa Lobo, 2001; Lin & Metters, 2006; Ritger & Peppas, 1987a). Theoretically, Eq. (3) is only valid for plane sheets with one-dimensional diffusion in a perfect sink. Whereas, many authors have employed it in systems in which one-dimensional flow cannot be assumed. Ritger and Peppas (1987a) stressed that deviations from one-dimensional flow and changes in environmental conditions affect the interpretation of the constant k and the transport exponent n. As observed in Table 2, in the case of spherical geometries, Fickian diffusion is defined by $n = 0.43$. When $n = 1$, the transport is named case II transport, where the rate of solvent absorption by the polymer is determined mainly by its swelling rate and/or the relaxation of its chains. Transport super case II ($n > 1$) is related to system plastification, which involves a reduction in the attractive forces between chains that increases mobility and facilitates the active compound release (Llabot et al., 2004).

Table 2
Transport exponent and mechanism of diffusional release from polymeric systems.

Transport exponent (n) of swellable systems	Transport exponent (n) of non-swellable systems	Transport mechanism	Rate as a function of time
0.43	0.43	Diffusion (Fick's Law)	$t^{-0.5}$
$0.43 < n < 1.00$	$0.43 < n < 0.85$	Anomalous transport	t^{n-1}
1.00	0.85	Case II transport	Zero order release
$n > 1.00$	$n > 1.00$	Super case II transport	t^{n-1}

Adapted from: Ritger and Peppas (1987a, b) and Costa and Sousa Lobo (2001).

Other models applied were the following:

i) Diffusion + relaxation (Peppas & Sahlin, 1989):

$$\frac{M_t}{M_\infty} = k_d t^m + k_r t^{2m} \quad (4)$$

M_t/M_∞ was defined in Eq. (3), k_d, k_r and m are constants. The first term represents the diffusional contribution and the second term represents the case-II relaxational contribution, taking into account the possibility of the two different mechanisms acting together.

ii) Burst effect (Huang & Brazel, 2001):

$$\frac{M_t}{M_\infty} = kt^n + \alpha \quad (5)$$

where k is a pre-exponential factor, n is the transport exponent and α is a constant added to fit experimental data when there is a fast increase in the release at initial times. This model assumes a fast initial release of compounds from capsules and then a time dependent release.

The different models described were applied to the first part of the kinetics, the time dependent zone. The R^2 values obtained by the statistical program were compared to select the model with the best fit to experimental data. Residual graphs were also analyzed by plotting the difference between the values estimated by the model and the means of the experimental values ($\hat{y}-\tilde{y}$) vs. the values estimated by the model ($\hat{y}$).

Based on the shape of the curve (Fig. 4b), the "burst effect" model (Eq. (5)) seemed to be the most suitable for dry capsules. According to Huang and Brazel (2001), active compound migration may occur during drying and storage. This process could lead to a heterogenous distribution of the active compound in the matrix and provoke a fast release at initial times. However, none of the proposed models fitted dry capsule kinetics. This fact was attributed to the rapid migration of polyphenols, where within the first 15 min almost 70% of the encapsulated mass was released.

Table 3 shows the fitting parameters obtained for wet capsules. Based on both criteria, (R^2 values and residual analysis), the diffusion model (Eq. (3)) showed the best fit for wet capsules. For diffusion model, an independent set of data was used to validate the model.

For both AY and ACHY capsules, the transport exponent (n) related to the type of release mechanism was below the value indicated for diffusion mechanism in Table 2. Only few authors found n parameters lower than 0.43. However, Ritger and Peppas (1987b) attributed an exponent value of 0.3 to the size distribution of microspheres. As observed in Fig. 3, capsules suffered erosion in simulated fluids, which could lead to an inhomogeneous size distribution during assay time. Grattard et al. (2002) found similar n values studying the release from microspheres of ethylcellulose obtained by spray-drying. These authors stressed that water solubility of the active compound, matrix porosity and affinity, strongly influence diffusive mechanism.

With respect to the diffusion + relaxation model (Eq. (4)), Table 3 shows that parameter k_d is higher than k_r for calcium alginate capsules. Therefore, the relationship between the two constants ($k_d > k_r$) suggests that the release mechanism is mainly diffusional. Whereas, in chitosan coated samples a negative value was found for k_d (Table 3). Probably, solubilization of chitosan in acidic media would also take place during active compound release, modifying the whole kinetic process.

In a previous work (Deladino et al., 2008), the diffusion model (Eq. (3)) was applied to the kinetic release of yerba mate polyphenols in water for wet capsules. The release mechanism was assigned to super case II ($n \approx 2$), where an initial chain

Table 3
Kinetic parameters obtained from release curves for wet capsules.

Sample	Diffusion model (Eq. (3))			Diffusion + relaxation model (Eq. (4))				"Burst release" model (Eq. (5))			
	k	n	R^2	k_d	k_r	m	R^2	k	n	α	R^2
AY	0.324	0.239	0.984	0.284	0.076	0.128	0.941	−0.927	−0.540	0.997	0.955
ACHY	0.384	0.308	0.937	−59.44	59.79	0.002	0.876	−1.426	−0.493	1.112	0.878

AY = Alginate capsules with yerba mate extract, ACHY = Calcium alginate–chitosan capsules with yerba mate extract.

plasticization allowed diffusion followed by a swelling and relaxation mechanisms acting together. However in gastric fluid, the drastic pH conditions led to capsule erosion and a faster release. The mechanism seemed to be mainly diffusive.

3.4. Enrichment of instant soups with encapsulated yerba mate extracts

The commercial soups have a mean polyphenols content of 0.3 mg of polyphenols/mL in prepared soup. Added soups (AYS and ACHYS) showed a value of 0.4 mg of polyphenols/mL in prepared soup. Control capsules treated with 250 mL of boiling water to simulate the soup preparation released 0.1 mg of polyphenols. This amount was the same that the difference found between soups with and without capsules. These results indicated that the extra amount of 0.1 mg of polyphenols incorporated by capsules in enriched soups, was not modified by the other soup components. Besides, after 7 months of storage no significant differences were detected in polyphenol content for both types of enriched soups.

3.4.1. Repose angle

Flow assurance is crucial to the design of bins or hoppers, especially to maximize the use of discharge units to their design capacity, in order to prevent costly downstream handling problems (Juliano, Muhunthan, & Barbosa-Cánovas, 2006). According to Peleg (1977), powders with repose angles lower than 40° are non-cohesive and powders with repose angles above 50° cause flow problems. Flow properties of enriched and commercial soups are shown in Fig. 5. The ANOVA showed that the type of soup, the storage time and the interaction between them were significant ($p < 0.05$) factors.

Fresh samples did not show significant differences ($\alpha = 0.5$, Tukey comparison test). Whereas, after 7 months of storage, the repose angle of soups with ACHY capsules was higher than commercial and AYS soup values. This fact was attributed to the morphology of chitosan coated capsules, which have an irregular shape (Deladino et al., 2008) that could lead to a higher friction between particles.

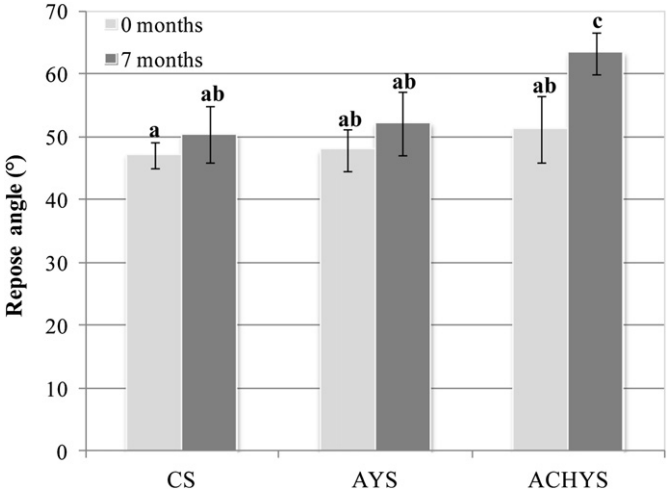

Fig. 5. Effect of storage time on the repose angle of fresh and stored enriched soups: CS = control soups, AYS = soups with AY (Alginate capsules with yerba mate extract), ACHYS = soups with ACHY (Calcium alginate–chitosan capsules with yerba mate extract). Different letters indicate significant differences between media values ($\alpha = 0.05$).

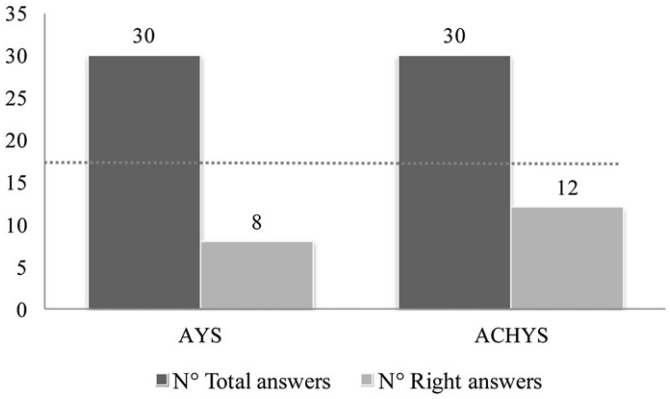

Fig. 6. Results of sensory analysis (Triangle test). AYS = soups with AY (Alginate capsules with yerba mate extract), ACHYS = soups with ACHY (Calcium alginate–chitosan capsules with yerba mate extract). Critical value (17) is represented by the dotted line ($\alpha = 0.01$; Ureña et al., 1999).

3.4.2. Sensory analysis

The triangle test has been widely used for detecting differences between samples for both research and applied works (Sauvageot et al., 2011). According to the number of panelists, the critical value was 17 for $\alpha = 0.01$ (Ureña et al., 1999). Since, only 8 panelists for AYS and 12 for ACHYS recognized the right answer, none of the enriched soups were significantly different from the commercial one (Fig. 6).

It is worth noting that yerba mate has a particular flavor and bitter notes. Thus, after the sensory results, it could be stated that encapsulation helped mask this typical taste not associated with these kind of soups.

4. Conclusions

Treatments of the capsules with sodium citrate or the whole model system (pH = 2 + pH = 7.4) were equivalent techniques to determine the maximum mass of loaded extract. The knowledge of the actual load is necessary to calculate the amount of polyphenols to be added in a food product.

Most of the encapsulated polyphenols were released in the simulated gastric fluid. Wet capsules coated with chitosan maintained a higher amount of active compound before reaching simulated intestinal fluid. Thus, ACHY capsules would be more suitable to extend and retain polyphenols inside the capsule.

Wet and dry capsules showed different release mechanisms. The wet capsules eroded in pH = 2 solution releasing their content mainly by diffusion; whereas, the dry ones easily hydrated and released most of the extract in acidic solution.

The addition of the encapsulated yerba mate extract to instant soups is a good alternative to increase their antioxidant content. Alginate capsules did not modify the flowability properties of the commercial soups. With the addition of encapsulated yerba mate, consumers will drink a healthier food with natural antioxidants without detecting any differences with commercial soups.

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