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Corn starch systems as carriers for yerba mate (*Ilex paraguariensis*) antioxidants

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A B S T R A C T

Corn starch's ability to bind and carry a *yerba mate* extract with strong antioxidant properties was explored in this paper. The starch was treated by high hydrostatic pressure to increase its loading capacity and scanning electron microscopy revealed that the high pressure treatment altered the shape of starch granules and changed their surface appearance. High pressure caused partial granule gelatinization as determined by calorimetric measurements, also increasing the granule specific surface area, as quantified by nitrogen adsorption. This increase in surface was due to the generation of pores, which favored the adsorption of the *yerba mate* hydrosoluble polyphenols. The *yerba mate* polyphenol concentration in the starch carriers was determined by HPLC-MS and its antioxidant activity was measured by the DPPH radical and photochemiluminiscense (PCL) methods. Treated starches incorporated a higher amount of *yerba mate* polyphenols, however, native starch could be an appropriate antioxidant carrier as well. Furthermore, it was found that the antioxidant activity was maintained after high pressure treatment without changing the *yerba mate* polyphenols profile.

Keywords: *Yerba mate*; Encapsulation; Corn starch; High hydrostatic pressure; Antioxidant activity; Specific surface area

1. Introduction

The availability, low cost and safety characteristics of native and modified starches and the new trends in functional food development broaden the spectrum of starch uses. Starch is both, a nutritional source and a commodity for the Food Industry. Encapsulation is one of the most promising techniques to carry bioactive compounds and allow their release at a target site. Rodrigues and Emeje (2012) summarized several studies on starch as an encapsulating material and Le Corre et al. (2010) reviewed the development and applications of starch nanoparticles.

The capacity of starch granules to carry bioactive compounds can be enhanced by expanding their intrinsic granule pores or generating new ones. Starch granules of diverse botanical origin (*i.e.*, corn, sorghum, millet, wheat) exhibit internal channels, many of which extend inward from the granule surface to the central cavity (Huber and BeMiller, 2000). In this respect, Kim and Huber (2013) prepared porous starch particles using citric acid and different time-temperature combinations to increase the load-carrying capacity of native corn starch granules. Also, amylolytic enzymes have been used to further increase granular porosity *via* limited erosion/hydrolysis of starch amorphous regions (Uthumporn et al., 2010).

High Hydrostatic Pressure (HHP) is a technology that opens several avenues to create materials with innovative functionalities, offering new possibilities to starch. Biopolymers,

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such as starch and proteins, undergo transformations of their native structure at high hydrostatic pressure comparable to modifications taking place at high temperatures (Stute et al., 1996). Depending on the pressure, temperature and duration of the HHP treatment, starch can gelatinize at lower temperatures compared with the traditional heat gelatinization, or even do it at room temperature (Stolt et al., 1999). Gelatinization kinetics and pasting properties could also be modified by high pressure, using different pH and osmolarity conditions (Simonin et al., 2011).

Considering that HHP acts almost instantaneously and independently from sample geometry and size, pressure-induced partially gelatinized starch would be more homogeneous than its thermal equivalent (Fernández et al., 2008). According to Knorr et al. (2006), during HHP starch gelatinization, starch granules remain intact or just partially disintegrated and amylose solubilization is rather poor. The stabilizing effect of the amylose still present in granules over the amylopectin is believed to partially protect starch crystallinity. During the HHP processing, new binding sites are generated and can be exploited to carry and deliver the molecules of interest, such as minerals (Fernández et al., 2008).

Yerba mate (*Ilex paraguariensis* Saint Hilaire) is a tree originating from South America that grows in a limited zone within Argentina, Brazil and Paraguay. Research on the effects of *Ilex paraguariensis* on health has confirmed its antioxidant, anti-inflammatory, antimutagenic and lipid-lowering activities (Bracesco et al., 2011). Considering the *yerba mate* known beneficial properties, several authors have carefully studied its composition and the main bioactive substances reported in *yerba mate* aqueous extracts were caffeic and chlorogenic acids and their derivatives (Anesini et al., 2012; Dugo et al., 2009; González de Mejía et al., 2009; Heck et al., 2008; Filip et al., 2001).

However, lyophilized *yerba* extract becomes a sticky material when it is not protected against ambient conditions (Deladino et al., 2007). Thus, the marked hygroscopic behavior that influences the flowability of this product needs to be minimized. Moreover, natural antioxidants should be protected from the surrounding medium or the processing conditions during food production (Fang and Bhandari, 2010).

Trends in replacing synthetic additives with natural ones and the high interest in nutraceutical foods led to focus on the possibility of vehiculization of *yerba mate* antioxidants in food systems. The objective of the work was to study the ability of native and high pressure treated corn starches to carry *yerba mate* extracts with antioxidant properties.

2. Materials and methods

2.1. Preparation of yerba mate extract

The active component was a lyophilized *yerba mate* extract (Y) obtained as described elsewhere (Deladino et al., 2008). Briefly, the extracts were obtained as follows: 1 g of commercial *yerba mate* (Las Marías, Corrientes, Argentina) was mixed with 100 mL of distilled water in a glass vessel, heated in a thermostatic bath (Haake, Germany) at 100 °C for 40 min. Then the extract was filtered, frozen and lyophilized at −80 °C for 24 h in a Heto FD4 Freeze Drying Machine (Allerod, Denmark). From 1 g of *yerba mate*, 0.327 g of lyophilized extract was obtained. Lyophilized extracts were stored in a dissecator in tightly closed flasks.

2.2. Preparation of starch carriers

Carrier systems were native corn starch (S) (Molinos Río de La Plata, Argentina) or high pressure treated starch (HPS). High pressure starches were obtained by suspending 10 g/100 mL of the aforesaid starch in deionized water (Milli-Q, Millipore Inc Bedford, MA, USA). A High Pressure Pilot Food Processor (Stansted Fluid Power LTD., Model FP 571000:9/2C, Harlow, UK) was employed, operating under 400 MPa, for 35 min with 38 °C as the initial vessel temperature and reaching a final process temperature of 40 °C. Starches were dried in an oven at 30 °C, powdered in a mortar and stored at room temperature in hermetic boxes.

2.3. Preparation of bioactive starches with yerba mate

Lyophilized *yerba mate* extract (1, 5 or 10 g) was added to starch aqueous suspensions (10 g/100 mL). Three types of products were obtained, depending on the sequence of extract addition and treatments:

HPSY*: the extract was dissolved in starch (S) suspensions and then, the resultant mixture was submitted to HHP treatment (*In situ* procedure), as described in section 2.2.

HPSY and SY: suspensions of HPS or S carriers were mixed with lyophilized *yerba mate* extract, placed in dark glass bottles and agitated in an orbital shaker (Orbit Environ Shaker, Lab Instruments, USA) at 25 °C and 180 rpm for 15 h (Immersion procedure).

All suspensions were centrifuged (Rolco, USA, 20 min, 300 × *g*) and the supernatants were discarded. Samples were dried in an oven at 30 °C and the obtained products were powdered in a mortar and stored in hermetic boxes. Table 1 summarizes the applied treatments and the samples nomenclature. Unless otherwise indicated, assays were performed in triplicate.

2.4. Characterization of bioactive starches

2.4.1. Scanning electron microscopy (SEM)

Scanning Electronic Microscopy (SEM) observation was performed with a FEI, Quanta 200 microscope (Eindhoven, Netherlands). Dried granules were attached to copper stubs using two-sided adhesive tape, then coated with a layer of gold (40–50 nm) in a vacuum evaporator and examined using an acceleration voltage of 20 kV.

2.4.2. Determination of the specific surface area

An accelerated surface area and porosimetry system was used (Sorptomat ASAP 2420, Micromeritics Inc., Norcross, USA) for nitrogen adsorption/desorption determination. Starch was degassed at 42 °C for 72 h. The adsorption isotherms were determined at the temperature of liquid nitrogen in the relative pressure (P/P_0) range of 0.05–0.12, taking 20 s to reach equilibrium. The specific surface area was calculated based on the BET adsorption isotherm. The pore size distribution was obtained applying the iterative method of Barret, Joyner and Hallendy (BJH) (Barret et al., 1951). Micropore area from t-plot was calculated at a P/P_0 of 0.2–0.5 for all samples, with the exception of native corn starch which was carried out at P/P_0 0.008–0.12. Assays were performed in duplicate.

2.4.3. Confocal laser scanning microscopy (CLSM)

Intact starch granules and cross-sections obtained by cryo-fracture were observed. Fluorescein isothiocyanate (FITC)

Table 1 – Nomenclature of the samples.

Yerba mate concentration (g/100 mL)	S (Native starch)	HPSY (Immersion)	HPSY* (*In situ*)
1	SY_1	$HPSY_1$	$HPSY^*_1$
5	SY_5	$HPSY_5$	$HPSY^*_5$
10	SY_{10}	$HPSY_{10}$	$HPSY^*_{10}$

(0.3 mg/mL) was used for labeling. Starch carriers (5 mg/mL) were suspended in Milli-Q water, then 1000 μL of starch suspensions were stained by the addition of 40 μL of FITC. The mixture was stirred in a vortex and allowed resting for 1 h in closed eppendorfs, in darkness and at room temperature, before analysis.

An inverted microscope (LEICA TCS SP5, Mannheim, Germany), equipped with an Ar and HeNe lasers, was used. The excitation and emission wavelengths were 488 nm and 518 nm, respectively. Images were acquired using a HCX PL APO CS 63.0 × 1.40/UV/oil immersion objective and with a 1024 × 1024 pixel resolution in a constant z-position. Software Leica Application Suite Advanced Fluorescence (LAS AF), version 2.2.1. build 4842 was employed in the image analysis.

2.4.4. *Differential scanning calorimetric studies*

Differential scanning calorimetric (DSC) determinations were performed with a DSC Q100 (TA Instruments, New Castle, USA), calibrated with an Indium standard, at a scanning rate of 10 °C min^{-1}, from 25 to 110 °C. Starch samples were dispersed in water (20 g/100 g) 24 h before DSC analysis. Then, samples were shaken and 15 μL of slurry were introduced in weighted aluminum pans, sealed and re-weighted. After analysis, pans were punctured and dried at 110 °C to constant weight. Thermograms were analyzed following standard procedures (Universal Analysis Program, TA Instruments, New Castle, USA). Analyses were performed at least in triplicate. Gelatinization enthalpy values (ΔH) were calculated from the endotherms and expressed in J/g sample on dry basis.

2.4.5. *Determination of polyphenol content in the yerba mate extract and in the bioactive starches*

Starch loaded with different *yerba mate* contents was subjected to several extractions with an adequate amount of Milli-Q water, depending on *yerba mate* concentration (Y_1 = 1 mL, Y_5 = 1.5 mL and Y_{10} = 3 mL). Aliquots were collected and mixed before determining polyphenol content and antioxidant capacity.

High performance liquid chromatography (HPLC) and mass spectrometry (MS) were used to identify the components of *yerba mate* extract and bioactive starches, based on their retention times and MS spectra, and by comparing them with pure standards and literature data. Analyses were performed using an Agilent 1100 series LC, comprised of a quaternary pump with integrated degasser, an autosampler, a thermostated column compartment and a diode array detector (DAD), coupled with an Agilent G1946D Quadrupole mass spectrometer (Agilent Technologies, Waldbroon, Germany). Data acquisition and analysis were carried out with an Agilent ChemStation Software. Samples of 20 μL were separated in a 150 mm × 4.6 mm i.d., 5 μm, C18 Agilent Zorbax Eclipse XDB-C18 analytical column (Agilent), eluted with a mobile phase of a mixture of deionized water (solvent A) and acetonitrile (solvent B), both containing 0.1% formic acid, at a flow rate of 1 mL min^{-1}. The solvent gradient changed according to the following conditions: from 90% A to 74% A in 40 min, to 35% A in 10 min, and then returning to the initial conditions in 5 min. Because of the lack of commercial standards, chlorogenic acid esters and isomers were quantified as chlorogenic acid.

Mass spectrometry data were acquired in the scan mode (mass range *m/z* 100–1100). Ions were produced by atmospheric pressure electrospray ionization (ESI). This source was operated in the negative ion mode, with the electrospray capillary voltage set to 3500 V, a nebulizing gas flow rate of 12 L/h, and a drying temperature of 350 °C. For negative polarity, *m/z* 179 (rutin), 353 (chlorogenic acid and isomers), 515 (chlorogenic acid esters) and 609 (caffeic acid) were used as standards. These compounds were quantified from the areas of their chromatographic peaks by comparison with calibration curves. Chlorogenic acid, caffeic acid and rutin standards were specific for HPLC assay, and their purity was ≥95% (Sigma, St. Louis, MO, USA). Analyses were performed in triplicate.

2.4.6. *Determination of antioxidant capacity*

2.4.6.1. Photochemiluminescence (PCL) free radical quenching assay. The *free radical quenching* inhibition capacity of starch carriers with and without *yerba mate* was assessed as described by Popov and Lewin (1996). This assay was carried out in a Photochem® instrument (Analytik Jena AG, Jena, Germany). The quenching of free radicals was visualized by means of the chemiluminescent reagent luminol (Balogh et al., 2010).

Aqueous samples obtained as described in 2.4.3 were analyzed using the Antioxidative Capacity of Water-soluble substances (ACW) kit. Pure compounds (chlorogenic and caffeic acids and rutin) were also tested as standards. Fresh samples of liquid extract were filtered through a syringe driven filter (Millex-GS, 0.22 μm, Millipore, USA) and then analyzed. Results were expressed as ascorbic acid equivalents. Analyses were performed at least in triplicate.

2.4.6.2. Radical scavenging ability toward DPPH free radical. Antiradical activity was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) (Sigma–Aldrich, Saint Louis, USA) as a free radical. The method was adapted from Brand-Williams et al. (1995) and it is based on the reaction of specific compounds or extracts with this radical in an ethanolic solution. DPPH• reduction was followed by measuring the absorbance decrease at a characteristic wavelength while the reaction occurs. The radical absorption disappears when it is reduced by an antioxidant substance or by other radical specie.

Starch samples were suspended in water, 100 mg/mL. A volume of 100 μL of each sample was added to 3.9 mL of DPPH• ethanol solution (25 mg DPPH•/mL ethanol). The decrease in absorbance was determined at 517 nm, until the reaction reached a plateau. Chlorogenic acid was also used as a standard.

The antioxidant activity of the extracts was expressed as the percentage of inhibition ($I_{DPPH^\bullet}$ %), which was obtained as follows:

$$I_{DPPH^\bullet}\% = \left[\frac{A_0 - A_s}{A_0}\right] \times 100 \quad (1)$$

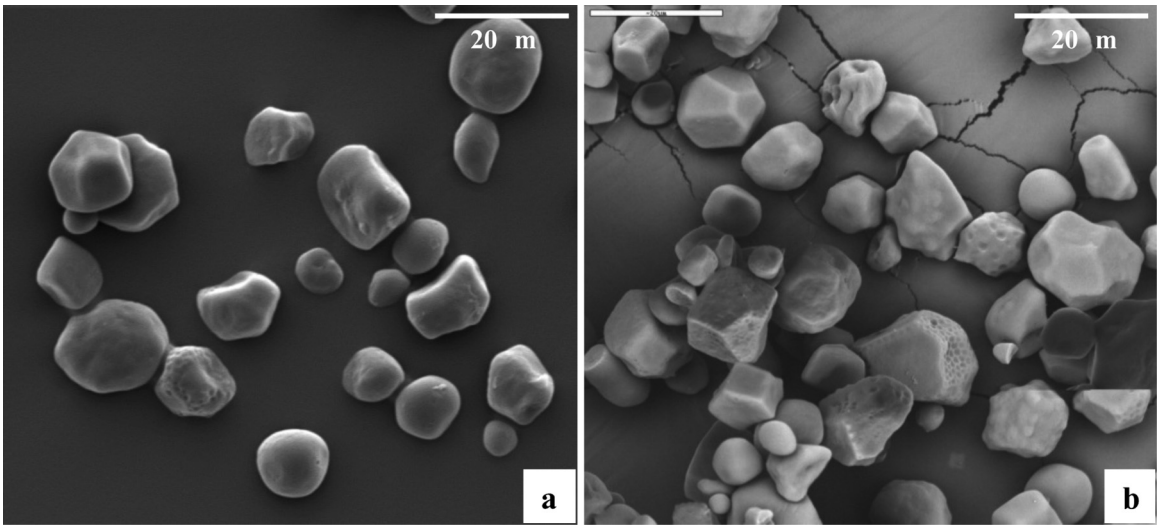

Fig. 1 – SEM photographs of (a) Native corn starch and (b) High pressure treated corn starch.

where A_0 was the initial absorbance of DPPH• solution and A_s, the absorbance of the sample after the plateau was reached. Analyses were performed at least in triplicate.

3. Results and discussion

3.1. *Starch microstructure*

High pressure treatment produced partial gelatinization evidenced by the starch granule morphology (Fig. 1). The smooth surface of native corn starch granules (Fig. 1a) became faceted and rougher with some external pores and hollows (Fig. 1b). Pei-Ling et al. (2012) observed similar changes in corn and tapioca starches treated with high pressure and stressed the formation of small cracks.

Fig. 2a shows the nitrogen adsorption/desorption isotherms for treated (HPS) and non-treated (S) starch and bioactive carriers with 5% of *yerba mate* (SY_5, $HPSY_5$ and $HPSY^*_5$). The type II isotherm found in all cases is characteristic of macroporous and non-porous adsorbents. For such materials, adsorption depends on the formation of a polymolecular layer on flat surfaces and inside pores. Additionally, if the adsorbent also contains mesopores, capillary condensation may take place (Włodarczyk-Stasiak and Jamroz, 2009). In the cases of $HPSY_5$ and $HPSY^*_5$, an important loop of hysteresis was observed (Fig. 2b). The size of the hysteresis area followed the order: $SY_5 < HPSY_5 < HPSY^*_5$. Juszczak et al. (2002) described several causes to explain this phenomenon. In the present work, the hysteresis could be attributed to both the increase of surface area due to the HHP treatment and the presence of *yerba mate* that could modify the pore affinity generating a new microenvironment.

The specific surface of starches can be employed as a measure of the surface activity which, in turn, could help evaluating the effect of the HHP treatment on the granules. This parameter depends on the size, shape and porosity of the granules. Besides, the specific surface area is proportional to the specific pore volume, and inversely related to the pore diameter (Juszczak et al., 2002; Sujka and Jamroz, 2007). Since corn starch granule size ranges from 2 to 30 μm (Fennema, 1996), the intra-particle pore analysis was focussed on a pore diameters lower than 60 nm. IUPAC (Sujka and Jamroz, 2007) classified pore sizes 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. After this classification, all the studied starch carriers had mesopores (Fig. 3a-b) The effect of high pressure treatment on pore diameter distribution of corn starch can be visualized in Fig. 3a. Native starch (S) showed a bimodal pore diameter distribution, with maximums around 5 and 17 nm (Fig. 3a). Average pore diameter for native corn starch was similar to those found by Juszczak et al. (2002). Treated starch (HPS) exhibited a decreasing pore size distribution from 1 nm. A difference in the shape of the pore distribution was also evidenced by an increase in the number of the smallest pores, due to the partial gelatinization induced by high pressure. The BET surface area was 0.277 m^2/g for native starch and 0.407 m^2/g for the treated starch, showing an increase of 47% after the HHP treatment.

Fig. 3b indicates that the addition of *yerba mate* by the immersion method did not affect the shape or the pore size distribution. However, when *yerba mate* extract was incorporated before HHP treatment, the distribution was slightly modified, showing a maximum at 3 nm without modifying the BET surface area (0.407 m^2/g) when compared to the HHP treated control.

Fig. 4 shows CLSM micrographs of native and HPS carriers. HPS samples showed a higher level of penetration of FITC dye (right column), related to a larger number of pores (Fig. 3). With respect to the granule shape, hollows and cracks can be seen (Fig. 4d), ascribed to partial gelatinization after the high pressure treatment, according to the SEM micrographs (Fig. 1). Internal cavities filled with FITC are clearly shown in HPS samples and, in some cases the dye reached the hilum (Fig. 4d and f).

According to Fernández et al. (2008), Knorr et al. (2006) and Błaszczak et al. (2005), the degree of gelatinization achieved depends on treatment conditions (pressure, temperature and time). Fannon et al. (1993) observed specific channels and cavities in corn starch granules and they suggested that surface pores are openings to serpentine channels penetrating into the granule interior. Huber and Bemiller (1997) also visualized these cavities and channels by dying starch granules with merbromim and van de Velde et al. (2002) visualized starch granule morphology using FITC. As was seen by porosimetry and CLSM results, high pressure partial gelatinization may favor connecting external pores with interior channels that were not accessible to nitrogen in untreated starch.

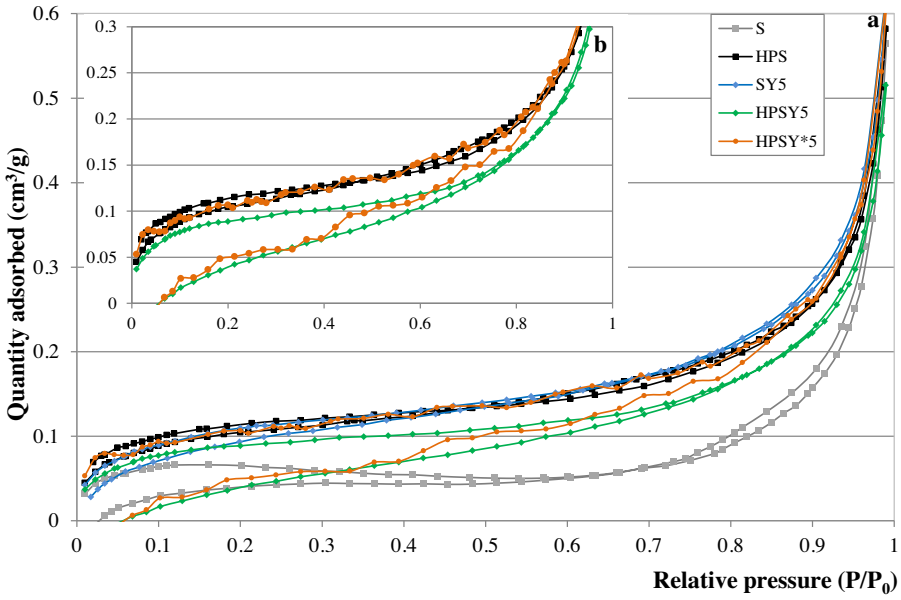

Fig. 2 – Nitrogen adsorption/desorption isotherms for (a) control corn starch (S), high pressure treated starch (HPS), and bioactive carriers with 5% of yerba mate (SY_5, $HPSY_5$ and $HPSY^*_5$) and (b) magnifications for HPS, $HPSY_5$ and $HPSY^*_5$ isotherms.

Fig. 5 shows DSC thermograms for native and HHP treated starch (controls) and bioactive starches with 5% of *yerba mate* loading. The gelatinization temperature for the HHP treated corn starch appeared slightly shifted toward lower values as compared to the native one. Also, gelatinization enthalpy decreased from 12 J/g to 9 J/g after the treatment. Thus, it was assumed that a 25% of the gelatinization degree took place during the applied HHP treatment. Buckow et al. (2007) and Liu et al. (2008) found similar results while studying the impact of high hydrostatic pressure, temperature and time on the gelatinization of different starches. In bioactive carriers, the addition of *yerba mate* did not affect gelatinization temperature, whereas, the decrease of enthalpy was around 6% for SY_5, 44% in the case of $HPSY_5$ and 67% for $HPSY^*_5$ compared with its respective controls (S or HPS). The decrease in enthalpy could be associated to a plasticizing effect of *yerba mate* extract polyphenols on starches. In a similar way, Cerruti et al. (2011) found that a polyphenol-containing extract derived from winery waste, used as a natural additive, exerted a plasticizing effect on Mater-Bi® (grade CF03A, Novamont, Italy), a starch-based polymer.

To sum up, HHP treatment provoked a partial starch gelatinization and increased its specific surface area. These

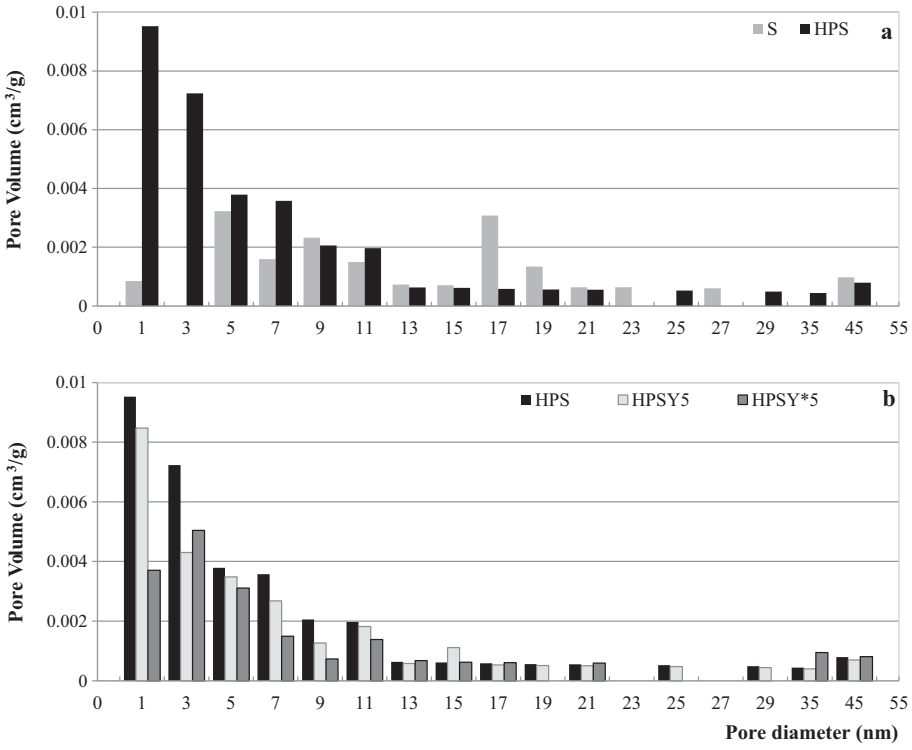

Fig. 3 – Effect of (a) HHP treatment and (b) yerba mate addition on pore volume distribution of corn starch. Control corn starch (S), high pressure treated starch (HPS) and bioactive carriers with 5% of yerba mate (SY_5, $HPSY_5$ and $HPSY^*_5$).

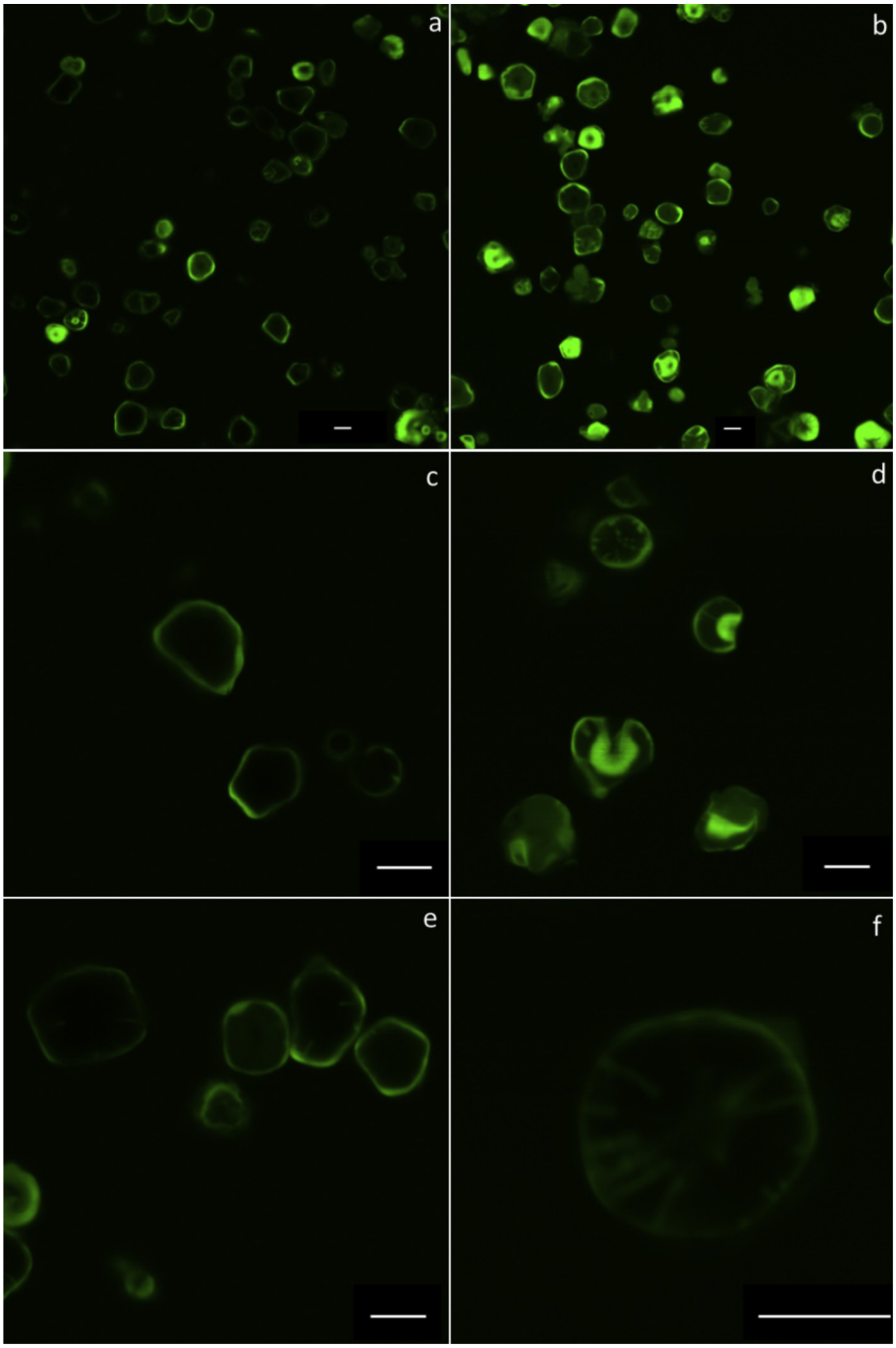

Fig. 4 – CLSM photomicrographs of corn starch granules (cross sections); left column: native starch, right column: high pressure treated starch. Bar = 10 μm.

modifications allowed an increased interaction with the active compound, which was evidenced by the modification of the shape of the adsorption curve, showing a higher hysteresis loop. These changes in granular structure due to the generation of pores could be advantageous for carrying active compounds such as *yerba mate* extract.

3.2. *Determination of polyphenol content in the yerba mate extract and the bioactive starches*

The characterization of *yerba mate* was based on its UV spectra and its mass spectral data. As it can be appreciated in Fig. 6a, main peaks at 280 nm were identified as chlorogenic acid (Rt = 5.2 min), caffeic acid (Rt = 7.3 min) and rutin (Rt = 12.2 min). Chlorogenic acid esters and isomers were the predominant compounds, as observed in the MS ion profile (Fig. 6b). These results match those obtained by other authors, such as Dugo et al. (2009), González de Mejía et al. (2009) and Filip et al. (2001).

The major polyphenols present in *yerba mate* extract were also detected in loaded starch systems. Total Polyphenol Content (TPC) of loaded starch products was calculated with HPLC–MS data, as the sum of individual polyphenol compounds contents. Fig. 7 shows that the loading of polyphenol extract was higher when products were obtained using HHP treated starches. This fact could be attributed to the higher porosity of these carriers, namely, the same starch mass with a higher amount of pores and void spaces loaded a higher content of the active compound. Even though, in the case of HPSY*, where *yerba mate* extract was added before the HHP treatment, the high pressure would favor solute diffusion into the granule.

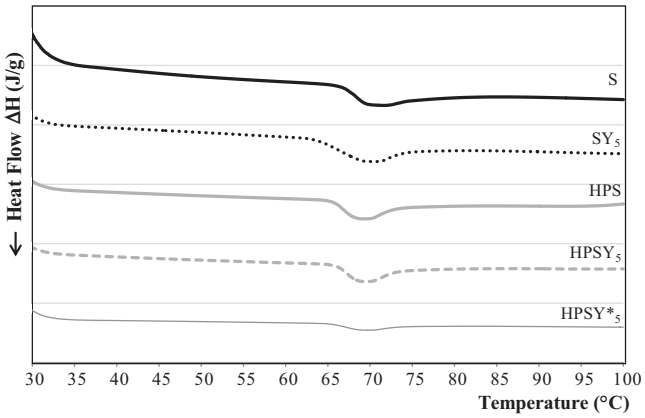

Fig. 5 – Gelatinization thermograms of control corn starch (S), high pressure treated starch (HPS) and bioactive carriers with 5% of yerba mate (SY_5, $HPSY_5$ and $HPSY^*_5$).

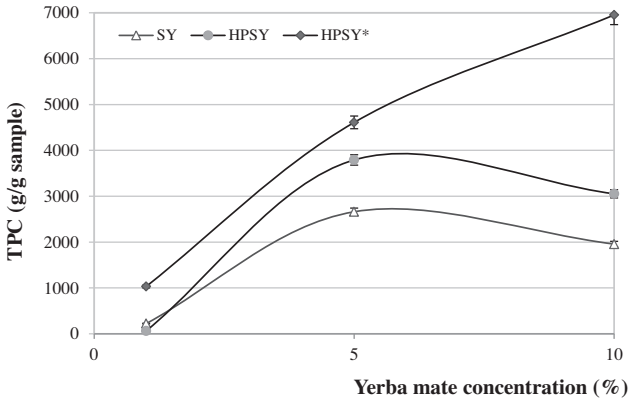

Fig. 7 – Effect of yerba mate concentration on the total polyphenol content (TPC) for corn starch carriers systems determined by HPLC-UV. SY = Non treated starch, HPSY = HP treated starch obtained by immersion and HPSY* = HP treated starch obtained *in situ*.

With respect to the effect of extract concentration on loading capacity, different increments in this capacity were observed between 1 and 5%, for all starch types (Fig. 7). Meanwhile, only the samples obtained *in situ* (HPSY*) showed a higher extract retention for a 10% extract concentration. Apparently, a saturation level was reached at 5% extract concentration for both untreated and immersion samples. However, the high pressure treatment in the presence of *yerba mate* polyphenols (HPSY*) allowed surpassing the saturation level reached under the previous conditions.

When a bioactive compound is encapsulated, it is desirable to attain the highest possible concentration. In the case of natural extracts, where several compounds are present, it is also important to determine if the proportion of each component has been altered with respect to the original extract. Preferential adsorption might have occurred in these starch carriers.

The contribution of individual phenolic compounds to the total polyphenol content of *yerba mate* extract and starch products is shown in Fig. 8. In all samples, chlorogenic acid esters and chlorogenic acid isomers are the major compounds, representing nearly 90% of the total. Rutin percentage was similar for all treatments and concentrations assayed, ranging between 3 and 6%. The three types of starch products showed a similar polyphenol profile for the 5% extract concentration.

Native starch and that resulting from immersion treatment showed a similar polyphenol profile for the 10% extract concentration. Chlorogenic acid esters, the extract compounds with the highest molecular weight, presented a lower

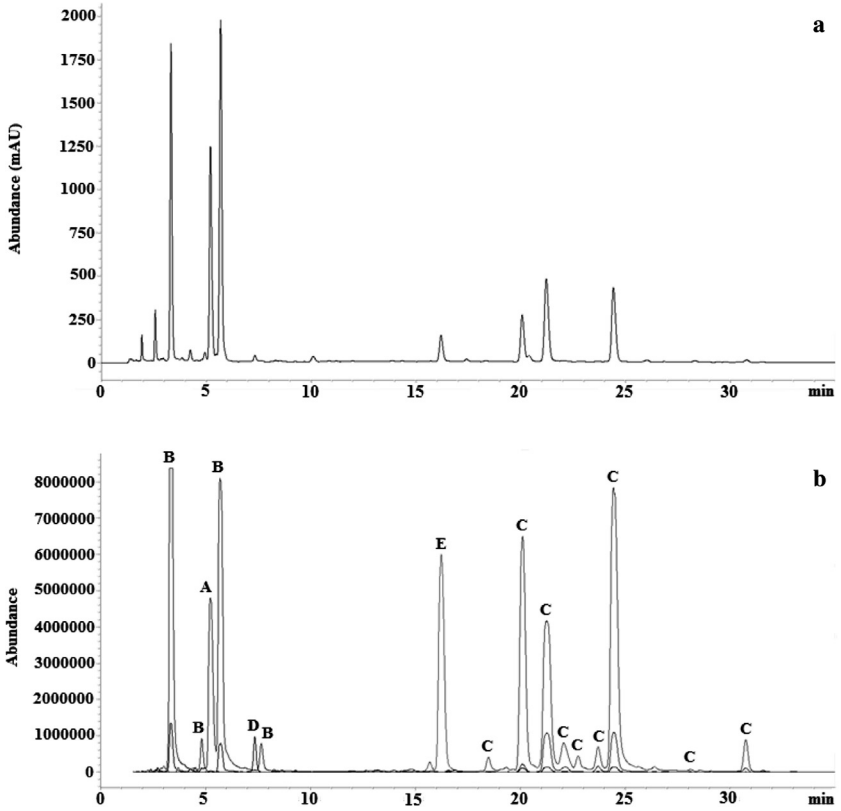

Fig. 6 – HPLC–UV chromatogram and total ion current profile of fraction in HPLC–MS. (a) UV chromatogram at 280 nm and (b) total ion current profile in MS, A = chlorogenic acid, B = chlorogenic isomers, C = chlorogenic esters, D = caffeic acid, E = rutin.

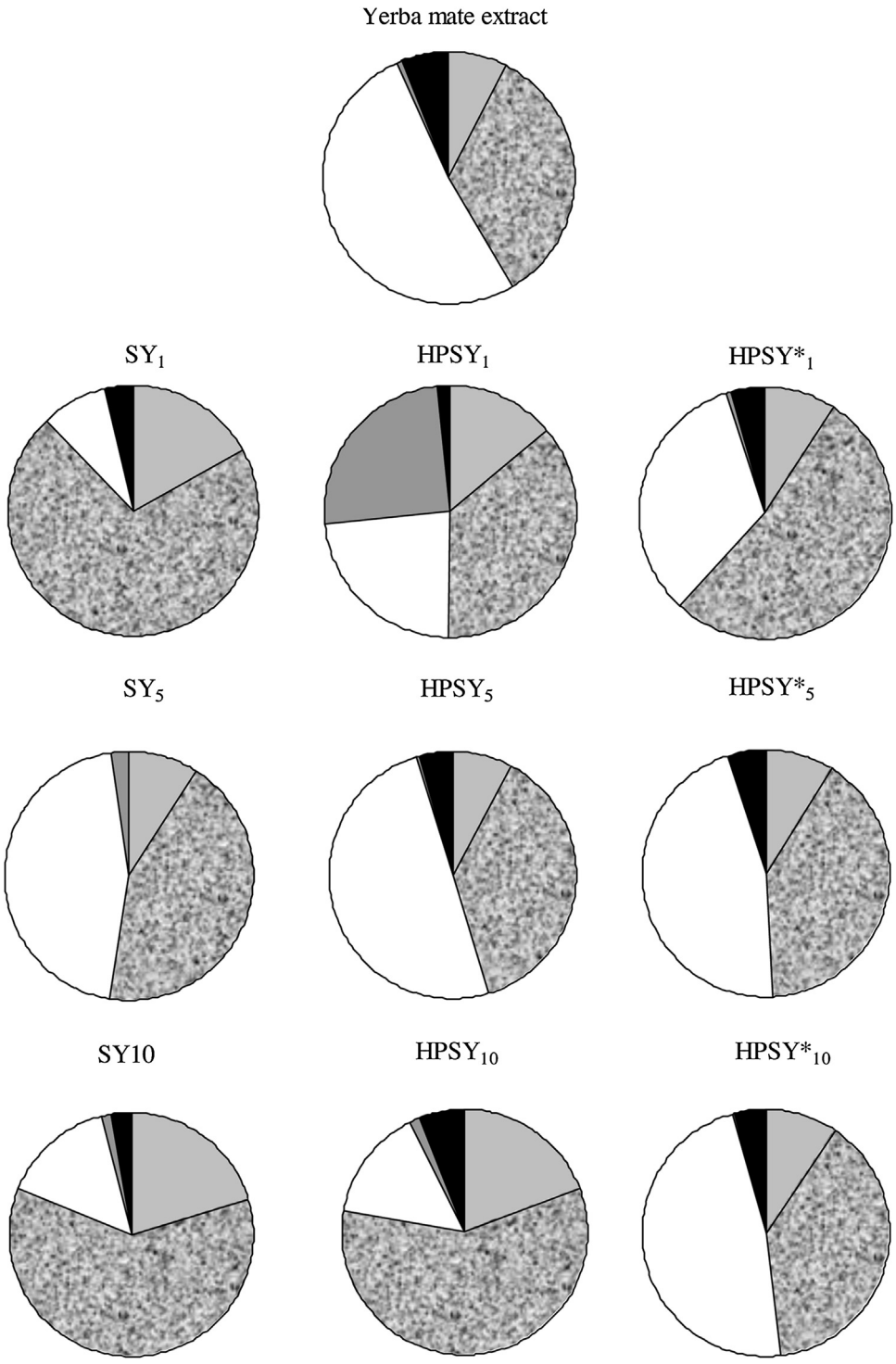

Fig. 8 – Contribution of each polyphenol compound to total polyphenol content of corn starches determined by HPLC–MS. SY = Non treated starch, HPSY = HP treated starch obtained by immersion and HPSY* = HP treated starch obtained *in situ*. CHAI (▨), CHAE (□), CHA (▤), CA (▥), Rutin (■).

contribution in these carriers. This fact might be attributed to preferential adsorption when reaching the saturation level (Fig. 8).

HPSY* was the carrier that best maintained the original polyphenol pattern of *yerba mate* extract. Even more, this pattern was independent of the extract concentration; this fact suggested that the high pressure treatment did not modify the extract composition, as compared with the other preparation conditions. Similarly, Patras et al. (2009) found that the polyphenol content of tomato and carrot purées did not change after HHP treatment.

3.3. *Antioxidant activity of carrier systems*

The antioxidant activity of the standards determined by PCL was approximately 4 mg equiv. ascorbic acid/g for chlorogenic acid and 10 mg equiv. ascorbic acid/g for caffeic acid, whereas, rutin did not contribute to the extract activity. Similar results were found by Anesini et al. (2012) when determining the antioxidant activity of pure compounds by DPPH•.

Antioxidant activity determined by both PCL and DPPH• techniques was not affected by the starch type (Fig. 9). The effect of extract concentration was reflected more properly by

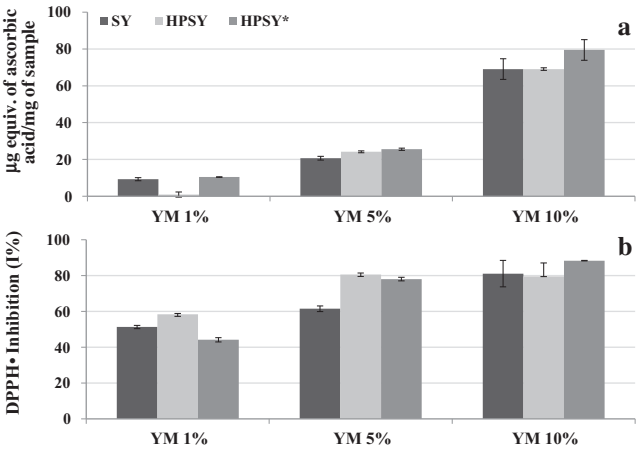

Fig. 9 – Antioxidant activity of corn starch systems for different yerba mate extract concentrations. (a) PCL determination and (b) DPPH• inhibition (%).

PCL. Fig. 9a shows that the antioxidant activity increased with *yerba mate* extract concentration, in good agreement with the total polyphenol content data, determined by HPLC-UV (Fig. 7).

In the case of the DPPH radical, no differences ($p > 0.05$) were found between samples with 5 and 10% of yerba mate extract content (Fig. 9b). This result was attributed to the DPPH• inhibition values obtained, which corresponded to the maximum inhibition percentage found for the crude yerba mate extract (around 90%). Patras et al. (2009) stressed that HHP treatments applied to plant samples do not modify their antioxidant activity. However, the activity of each product should be tested, according to Oey et al. (2008), the effect of high pressure processing on antioxidant capacity depends on the treated food.

The TPC results determined by HPLC showed the effect of the starch type carrier, procedure type (immersion or *in situ*), as well as the extract content. However, the antioxidant capacity measurement techniques applied in this study did not significantly register these differences. On the other hand, PCL was able to detect the effect of *yerba mate* concentration.

4. Conclusions

Corn starch was suitable as a carrier for a *yerba mate* extract, maintaining its antioxidant power. The treatment by high hydrostatic pressure improved the starch ability to carry bioactive compounds, an effect attributed to the development of new pores within granules. Nevertheless, untreated starch also showed the capacity to transport *yerba mate* extracts.

High pressure *in situ* treatment captured the highest polyphenol content. Besides, the polyphenol profile was not affected by the high pressure treatment, regardless of the initial concentration of the *yerba mate* extract.

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