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Yerba mate antioxidant powders obtained by co-crystallization: Stability during storage

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ABSTRACT

The interest on yerba mate has increased in the last years due to its high content of bioactive compounds related to health benefits. Antioxidant aqueous extracts of yerba mate were entrapped into a sucrose matrix by co-crystallization. The products were characterized in terms of their morphology, entrapment yield, loading capacity, water activity, moisture content, antioxidant activity, thermal behavior, solubility and hygroscopicity. It was found that the co-crystallization process led to high entrapment yield and maintained the antioxidant activity of the yerba mate extract towards DPPH radical. Also, the co-crystallized powders showed values of water activity, moisture content, hygroscopicity and flowability indicative of high stability and good handling properties. The total polyphenols content of the co-crystallized products remained almost constant along storage at 75% RH and 20 °C, however, fluctuations in their DPPH radical scavenging activity were observed.

1. Introduction

Yerba mate (*Ilex paraguariensis*) is highly consumed as infusion in several South American countries and has been recognized by FDA as GRAS (generally recognized as safe). The extract of yerba mate could be considered as a natural antioxidant to preserve food by retarding deterioration, rancidity, or discoloration and also, has the advantage of being effective at very low concentrations (De Campos et al., 2007; Valerga et al., 2013). Various pharmacological activities inherent to the yerba mate extract have been attributed to their content of polyphenols, flavonoids and xanthines including caffeic acid, chlorogenic acids, rutin, caffeine and theobromine (Anesini et al., 2012; Bravo et al., 2007; Jaiswal et al., 2010). Recent studies have demonstrated that the phenolic compounds act in the prevention and treatment of health disorder due to its antioxidant, hepatoprotective, choleretic, diuretic, hypocholesterolemic, anti-rheumatic, antitrombotic, antiinflammatory, antiobesity and anti-ageing properties (Bracesco et al., 2011; Marques and Farah, 2009).

Natural antioxidants may be added to a wide range of food such as baked goods, biscuits, chewing gum, dry snacks, fruit drinks, mayonnaise, meat products, nuts, oils and fats, among others. However, it is well known that the aqueous extracts obtained from plants, exhibit low stability and some of them have an unpleasant flavor, therefore, only a few extracts are currently employed in the food industry (Kosaraju et al., 2008; Makris and Rossiter, 2000; Wanasundara and Shahidi, 2005).

Encapsulation technologies become an actual choice applied to preserve and/or protect numerous ingredients from adverse environmental conditions (light, moisture, and oxygen) and to prevent undesirable interactions with the carrier food matrix (Gouin, 2004; Onwulata, 2011). Recently, Fang and Bhandari (2010) and Munin and Edwards-Lévy (2011) published interesting reviews focused on the techniques employed for the encapsulation of polyphenols such as spray drying, coacervation, liposome entrapment, inclusion complexation, co-crystallization, nanoencapsulation, freeze drying and emulsion. Spray drying is the most widely applied process mainly for the preparation of dry, stable food additives and flavors (Desai and Jin Park, 2005). Co-crystallization offers an economic and flexible alternative for the incorporation of active compounds into powder foods. In this process, the crystalline structure of sucrose is modified from perfect to irregular agglomerated crystals, to provide a porous matrix in which a second active ingredient can be incorporated. These agglomerates have a sponge-like appearance, with considerable void space and an increased surface area (Awad and Chen, 1993; Chen et al., 1988). Co-crystallization with sucrose could improve the solubility, dispersibility, wettability, anticaking, antidusting, antiseparation, homogeneity, flowability and stability of food materials. Nevertheless, few studies have been carried out on this technique dealing with flavors, natural extracts, essential oils, honey, glucose and fructose (Astolfi-Filho et al., 2005; Beristain et al., 1996; Bhandari et al., 1998; Bhandari and Hartel, 2002; Maulny et al., 2005; Sardar and Singhal, 2013).

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Previously, we have obtained co-crystallized products working with mineral salts and freeze-dried yerba mate extract which results demonstrated that the co-crystallization was a good alternative for the handling of these materials (Deladino et al., 2007, 2010). Further studies are necessary for co-crystallization to reach industrial applications. In this regard, simplifications of the co-crystallization steps would favor scaling up of the process. In the present work, an improvement in the production of the co-crystallized powders was carried out: the aqueous extract of yerba mate was employed as the solvent of the supersaturated sucrose solution. The physicochemical characterization of the antioxidant powders was performed and their stability during storage was evaluated, as well.

2. Materials and methods

2.1. Preparation of the yerba mate extract

Different amounts (3, 5 and 10 g) of commercial yerba mate (Las Marías, Corrientes, Argentina) were mixed with 100 mL of distillated water and 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.

2.2. Preparation of the co-crystallized products

A blend of commercial sucrose (50 g) (Ledesma, Argentina) and yerba mate extract (10 mL) was heated to 132 °C on a hot plate and stirred with a vertical agitator (IKA Labortechnik, Staufen, Germany). When a slight turbidity was detected in the syrup, indicating the beginning of crystallization process, the mix was removed from the heat, maintaining the agitation. Then, co-crystallized products were dried in a convection oven (SanJor, Argentina) at 40 °C for 15 h, milled and transferred to polyethylene bags for storage in desiccators with silica gel until use. Three batches of co-crystallized powders were prepared as described above. A supersaturated sucrose solution without the active compound was processed in the same manner as described above to act as a control. Agglomerates were sieved with a set of meshes between 2 and 0.250 mm. Size distribution of the agglomerates was calculated by the weight of the powder retained in each sieve. Specifically, the particles retained in the meshes of 0.5 and 1 mm were used to determine the solubility, flowability, hygroscopicity and the chemical stability during storage.

2.3. Total polyphenols content

Total polyphenols content (TPC) was determined by the Folin–Ciocalteu method (Singleton et al., 1999). Briefly, 2 mL of Na_2CO_3 (2 g/100 mL) (Anedra, Argentina) were mixed with 200 μL of the sample and 200 μL of Folin–Ciocalteau reagent (Anedra, Argentina, 1:1 diluted). After 30 min, sample absorbance was measured at 725 nm in a spectrophotometer (Shimadzu, UV-mini 1240, Japan). Chlorogenic acid (Fluka, USA) was used as standard.

2.4. Loading capacity and entrapment yield

Loading capacity (L_c) was calculated as the TPC of the yerba mate extract loaded in 1 g of co-crystallized material. The entrapment yield (%EY) was calculated as follow:

$$\%EY = \left(\frac{L_c}{L_0}\right) \times 100 \quad (1)$$

where L_0 is the initial TPC of the yerba mate extract per gram of raw mix.

2.5. Antioxidant activity of the yerba mate co-crystallized powders

The antioxidant activity was determined measuring the free radical scavenging activity towards the 1,1-diphenyl-2-picrylhydrayl reagent (DPPH·) (Sigma–Aldrich, USA) according to the method described by Brand-Williams et al. (1995). One gram of co-crystallized product was dissolved in 10 mL of distilled water. An aliquot of 100 μL of dissolution was 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 percentage of inhibition (I%) of the DPPH· free radical, calculated with the following equation:

$$I(\%) = \left(\frac{Abs_b - Abs_s}{Abs_b}\right) \times 100 \quad (2)$$

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

2.6. Characterization of the co-crystallized products

2.6.1. Scanning electronic microscopy (SEM)

Morphological analysis was performed by SEM using a FEI, Quanta 200 microscope (Netherlands). Samples 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.

2.6.2. Color attributes

Color was measured using a tristimulus Minolta colorimeter (Konica-Minolta CR-400, Japan) and was reported in CIELab scales (L^*, a^* and b^* values), where L^* was used to denote lightness, a^* redness and greenness, and b^* yellowness and blueness. Chroma and hue angle values were calculated using Eqs. (3) and (4), respectively.

$$\text{Chroma} = [a^{*2} + b^{*2}]^{1/2} \quad (3)$$

$$\text{Hue angle} = \tan^{-1}(b^*/a^*) \quad (4)$$

2.6.3. Moisture content and water activity

Moisture content (%) was measured gravimetrically by drying the grounded samples in a vacuum oven at 70 °C, until constant weight (AOAC, 1998). Values of water activity (a_w) were determined using an AquaLab Serie 3 TE (USA) equipment.

2.6.4. Solubility

Solubility was determined by blending 1 g of co-crystallized powder with 10 mL of distilled water at ambient temperature with continuous stirring (IKA RH1 magnetic stirring, Germany). Aliquots were removed at different times and the dissolved sucrose mass in the solution was determined using a HI96801 digital refractometer (Hanna Instruments, USA).

2.6.5. Flowability tests

Flowability of the co-crystallized powders was determined by both, dynamic angle of repose and Hausner ratio (H). The angle of repose was determined with a rotating cylindrical chamber, which was tilted gradually until slipping occurred and the angle measured (Solids handling study bench, CEN, Armfield, United Kingdom) (Geldart et al., 2006).

The value of H was calculated by the ratio of the tap bulk density to the loose bulk density (Hausner, 1967). The loose bulk density was determined by pouring a known mass of co-crystallized product delivered freely by gravity into a measuring cylinder, and it was calculated by dividing the mass by the bulk volume.

The tap bulk density was calculated from the weight of powder contained in the cylinder after being hand tapped 100 times at roughly 60 taps/min (Pordesimo et al., 2009). All measurements were performed at least in triplicates.

2.6.6. Differential scanning calorimetry (DSC)

The equipment used was a DSC Q100 (TA Instruments, USA), calibrated with an Indium standard. Samples of 3–5 mg were placed in aluminum pans hermetically sealed and an empty pan was used as the reference. Samples were heated from 25 °C to 250 °C at a heating rate of 10 °C/min.

2.6.7. Fourier transform infrared spectroscopy (FT-IR)

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

2.6.8. Water gain, hygroscopicity and chemical stability during storage

Petri dishes filled with the co-crystallized powders were placed in hermetically sealed glass desiccators containing supersaturated solution of NaCl (75% RH), and stored at 20 °C. The samples were removed out in different times and its weight gain was determined until constant value. Hygroscopicity (HG%) was expressed as the final moisture content attained after exposing the powder under the conditions mentioned above. HG% values were calculated from modified Jaya and Das (2004) equation to calculate water gain values on dry basis as follows:

$$HG(\%) = \left(\frac{b+H}{a-H}\right) \times 100 \quad (5)$$

where b (g) is the weight increase, a (g) is the initial sample weight and H is the initial water content of the powder (g).

The chemical stability was monitored through the total polyphenols content and the antioxidant activity for 120 days. Once the samples reached the equilibrium water content, they were maintained under the same conditions and analyzed at different times.

High performance liquid chromatography (HPLC) was employed to determine the major phenolic compounds and caffeine in the co-crystallized yerba mate extract only before and after 120-day storage. The analysis were done using an HP 1100 liquid chromatograph (Hewlett Packard, US) equipped with a binary pump, a thermostated column compartment, auto injector, degasser and diode-array detector (DAD) connected to an Agilent workstation. A Zorbax 300 SB-C18 column (4.6 × 250 mm; 5 µm) connected to a guard column was used. A modified method from Chandra and Gonzalez de Mejia (2004) was carried out as follows: the mobile phases "A" and "B" consisted of a mixture of water, methanol and formic acid (79.7/20/0.3) and a mixture of methanol and formic acid (99.7/0.3), respectively. A staggered gradient elution program at 0.9 mL/min prepared as follows: 0% B/15 min, 40% B/15 min; 75% B/10 min; 100% B/5 min, was employed. Commercial standards (Sigma–Aldrich, Argentina) of chlorogenic acid, caffeic acid, rutin and caffeine were used. Stock solutions of each standard (0.25 mg/mL) were prepared in 50% methanol-milli-Q water. Calibration curves at four different concentration levels were performed. Each level was tested by duplicate. The retention times (t_R) and DAD absorbance spectral matching were used for identification purposes. Based on the absorption maxima, the wavelengths selected for each compounds were 275 nm for caffeine, 330 nm for chlorogenic and caffeic acids and 360 nm for rutin.

2.7. Statistical analysis

The data analysis was performed with the software SYSTAT INC. (Evenston, USA. Analysis of variance (ANOVA) and mean comparisons were carried out. Unless indicated, a level of 95% of confidence ($\alpha = 0.05$) was used.

3. Results and discussions

3.1. Entrapment yield, loading capacity and antioxidant activity of the co-crystallized products

Antioxidant co-crystallized powders were obtained from the liquid extracts prepared with 3, 5 and 10 g of yerba mate/100 mL and called CC-YM3, CC-YM5 and CC-YM10, respectively. Values of entrapment yield of around 85% were obtained in all cases, even working at high temperatures such as 132 °C. Deladino et al. (2007) working with co-crystallized freeze-dried yerba mate extract obtained an average yield of 72%. In the present study, the use of liquid extract instead of freeze-dried powder increased the yield of the process; and it could also have improved the active compound distribution in the matrix. Fig. 1 shows a relationship between the loading capacity and the antioxidant activity towards DPPH· radical of the co-crystallized products. ANOVA showed that the polyphenols concentration of the raw yerba mate extract was a significant factor for the loading capacity of co-crystallized powders ($p < 0.05$). In this sense, the highest value of Lc was obtained for CC-YM10. This parameter is important for calculating the mass of yerba mate powders necessary to reach a determined polyphenols level in a food formulation. Besides, an increase in the antioxidant activity (DPPH· inhibition percentage) was observed with increasing loading capacity of yerba mate polyphenols (Fig. 1).

In order to compare the measured DPPH· inhibition (%) with the expected value for the liquid extract, a linear correlation between the DPPH· inhibition (%) and the polyphenols content of free (non-encapsulated) yerba mate extract was obtained ($R^2 = 0.98$). The expected DPPH· inhibition percentages were extrapolated using the actual polyphenol content of the co-crystallized products. Similar values of expected and measured DPPH· inhibition percentages were obtained (Fig. 1). Thus, these results indicated that the co-crystallization process did not modify the antioxidant activity of the yerba mate extract.

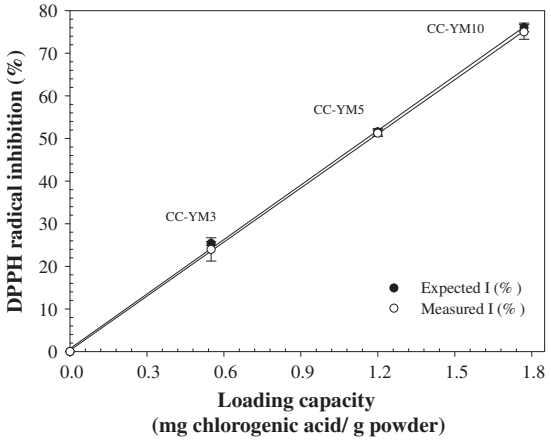

Fig. 1. Loading capacity vs. DPPH· inhibition percentage of the co-crystallized powders. I%: Percentage of inhibition towards DPPH· free radical.

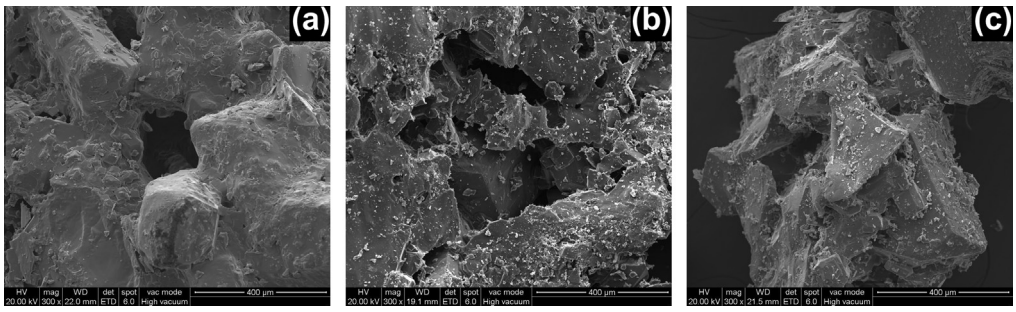

Fig. 2. SEM micrographs of (a) control co-crystallized powder and (b and c) CC-YM3 and CC-YM10 yerba mate co-crystallized powders, respectively.

Table 1
Color attributes of control and yerba mate co-crystallized products.

Sample	Color attributes		
	L	Chroma	Hue angle (°)
CC-C	64.4 ± 4.2	2.3 ± 0.6	95.8 ± 1.2
CC-YM3	55.3 ± 5.3	6.9 ± 1.6	99.3 ± 1.6
CC-YM10	55.9 ± 2.2	11.0 ± 0.8	94.4 ± 0.4

CC-C: control co-crystallized powder; CC-YM3 and CC-YM10: yerba mate co-crystallized powders.

Table 2
Flowability of control and yerba mate co-crystallized products with different particle size.

Sample	Repose angle (°)		Hausner ratio	
	0.5 mm	1 mm	0.5 mm	1 mm
CC-C	41.8 ± 4.7	41.8 ± 4.9	1.02 ± 0.04	1.10 ± 0.02
CC-YM3	40.4 ± 4.4	43.2 ± 4.8	1.03 ± 0.02	1.01 ± 0.02
CC-YM10	40.4 ± 2.6	43.2 ± 5.4	1.02 ± 0.02	1.02 ± 0.02

CC-C: control co-crystallized powder; CC-YM3 and CC-YM10: yerba mate co-crystallized powders.

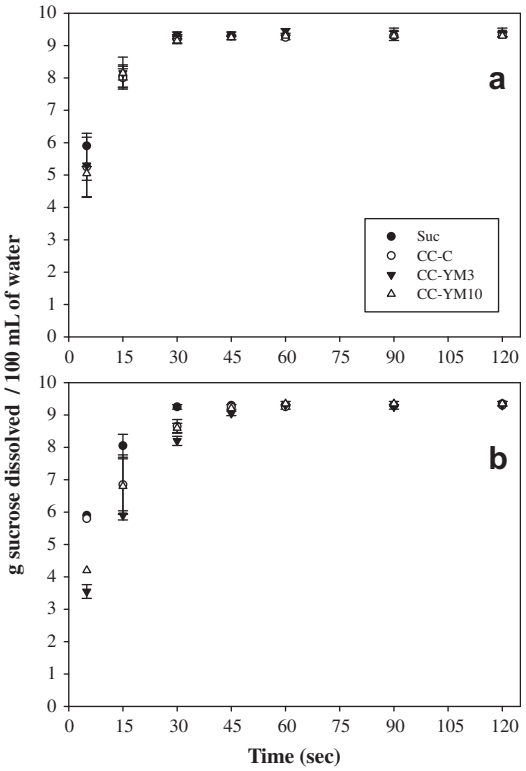

Fig. 3. Dissolution kinetics of sucrose raw (Suc), control (CC-C) and yerba mate co-crystallized powders (CC-YM3 and CC-YM10): (a) powders retained in a mesh of 0.5 mm and (b) powders retained in a mesh of 1.0 mm.

To analyze the effect of the polyphenols concentration, the co-crystallized products CC-YM3 and CC-YM 10 were selected to carry out the characterization experiments and the storage studies.

3.2. Physicochemical properties of the co-crystallized powders

The co-crystallized products of yerba mate presented a wide distribution of particle sizes (between 0.2 and 2 mm). However,

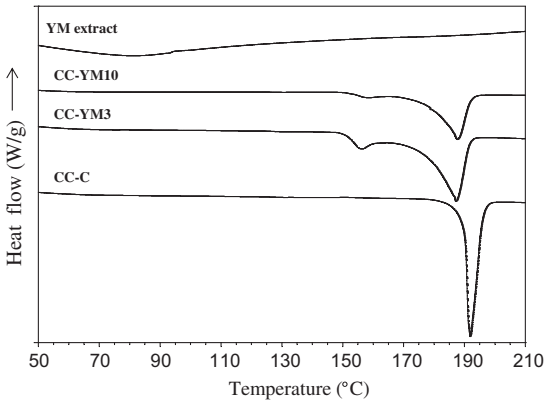

Fig. 4. DSC thermographs for control (CC-C), yerba mate co-crystallized powders (CC-YM3 and CC-YM10) and lyophilized yerba mate extract (YM extract).

the higher amount of particles (around 75%) was retained within 0.5–1.0 mm, whereas the agglomerates with particle higher that 1 mm accounted only for 1%. A coarse material was obtained according to British Pharmacopoeia that classifies the powder materials by their particle size distribution as: coarse, moderately fine, fine and very fine (British Pharmacopoeia, 2012). The particle size distribution is a relevant factor for the processability of powder materials (flow, mixing and compaction) and also for the final characteristics of the products (consistency, appearance and stability) (Barbosa-Cánovas et al., 2005).

SEM micrographs of the control and yerba mate co-crystallized products showed a characteristic porous structure corresponding to cluster-like agglomerates with irregular cavities between them (Fig. 2). Similar observations were reported with other co-crystallized products (Bhandari and Hartel, 2002; Chen et al., 1988; Deladino et al., 2010).

Color attributes of the powders were significantly affected by the concentration of the yerba mate extract (Table 1). A decrease in the lightness of the products with the incorporation of the extract was observed. The chroma values increased with the amount

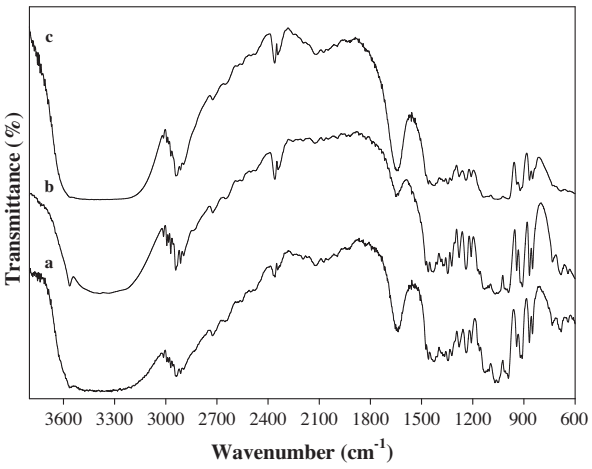

Fig. 5. FT-IR spectra of (a) control co-crystallized powder and (b and c) CC-YM3 and CC-YM10 yerba mate co-crystallized powders, respectively.

Table 3
Hygroscopicity (HG%) of co-crystallized products at 75 %RH and 20° C.

Sample	HG (%)	
	0.5 mm	1 mm
CC-C	0.2 ± 0.01	0.2 ± 0.01
CC-YM 3	0.3 ± 0.04	0.3 ± 0.10
CC-YM10	0.3 ± 0.10	0.3 ± 0.10

CC-C: control co-crystallized powder; CC-YM3 and CC-YM10: yerba mate co-crystallized powders.

of yerba mate extract loaded in the powders. Similar values of Hue angle were obtained for all the samples. These values were characteristic of green–yellow color of yerba mate extract.

The co-crystallized powders showed a_w and water content lower than 0.5% and 0.3%, respectively. These values are favorable to prevent the spoilage of the encapsulated active compounds during storage. In sugar-rich foods with a_w below 0.6 no microbial proliferation occurs and the product could be considered fully stable in that respect (Fennema and Tannenbaum, 1996; Fu and Labuza, 1993).

The kinetics of dissolution of sucrose (Suc), control (CC-C) and yerba mate extract co-crystallized products (CC-YM3 and CC-YM10) are shown in Fig. 3. A high dissolution rate for the co-crystallized products was observed, non-significant differences ($p > 0.05$) were obtained compared to sucrose or control co-crystallized regardless of the particle size. A dissolution time of about 45 s was obtained for all samples, over this time non-significant changes in the sucrose mass determined by refractometry were observed and no solid particles were visualized. According to Chen et al. (1988) the co-crystallized active compounds are located primarily in the interstices between crystals. Due to the porosity of the agglomerates, aqueous solution can rapidly penetrate the agglomerate and free the active compound for dissolution.

The flow properties are useful to predict the powder handling during production and the caking characteristics during storage (Geldart et al., 2006; Santomaso et al., 2003). In the present work, the dynamic angle of repose and the Hausner index were used to determine the flowability of the co-crystallized products (Table 2). The particle size and the concentration used of the yerba mate extract did not significantly modify ($p > 0.05$) the flow properties of the materials. Values of repose angle of around 42° were obtained for all the analyzed samples. Similar results were reported for co-crystallized products with mineral salt (Deladino et al., 2007) and honey (Bhandari et al., 1998). Several author have reported that materials with repose angles between 40° and 45° are free-flowing and powders with repose angles above 50° are very cohesive and could cause handling problems (Antequera et al., 1994; Peleg, 1977). Also, Hausner ratio values of around 1.0 were obtained in all cases. According to reported literature data a Hausner ratio less than 1.2 is indicative of a good flowability, whereas a value of 1.5 or higher suggests a poor flow display by the material (Geldart et al., 2006; Santomaso et al., 2003).

Fig. 4 shows the DSC thermographs obtained for the control (CC-C), yerba mate co-crystallized powders (CC-YM3 and CC-YM10) and freeze-dried yerba mate extract (YM extract). The yerba mate extract showed a broad peak at 85 °C, the endotherm transition shape is probably due to the great diversity of compounds present in the extract. CC-C showed an endothermic peak around 191.7 °C typical of sucrose melting. Similar temperatures were reported by Bhandari and Hartel (2002) and Sardar and Singhal (2013). In the thermographs of CC-YM3 and CC-YM10 the melting peak assigned to sucrose shifted to lower temperatures (around 187.5 °C); and an endotherm around 156.5 °C was observed, as well. Similarly, Beckett et al. (2006) reported peaks at 192 and 150 °C in the thermograph of crystalline sucrose, the peak at 150 °C was found to be highly dependent on the presence of other compounds in sucrose, especially in terms of the mineral salt content.

On the other hand, no second-order phase transitions were observed in the thermographs of the co-crystallized powders (Fig. 4), indicating that after the process of co-crystallization the sucrose maintains its crystalline structure. Besides, the disappearance of the YM extract peak at 85 °C could indicate that the co-crystallization with sucrose increase the thermal stability of the yerba mate

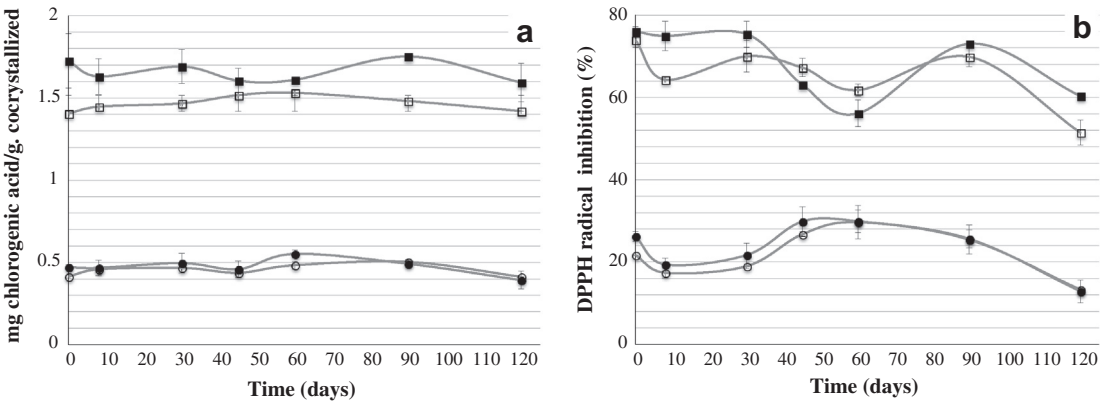

Fig. 6. Stability of co-crystallized yerba mate extract during the storage: (a) Total polyphenols content and (b) Antioxidant activity towards DPPH·. (o) CC-YM3 retained in a 0.5 mm mesh, (●) CC-YM3 retained in a 1 mm mesh, (□) CC-YM10 retained in a 0.5 mm mesh and (■) CC-YM10 retained in a 1 mm mesh.

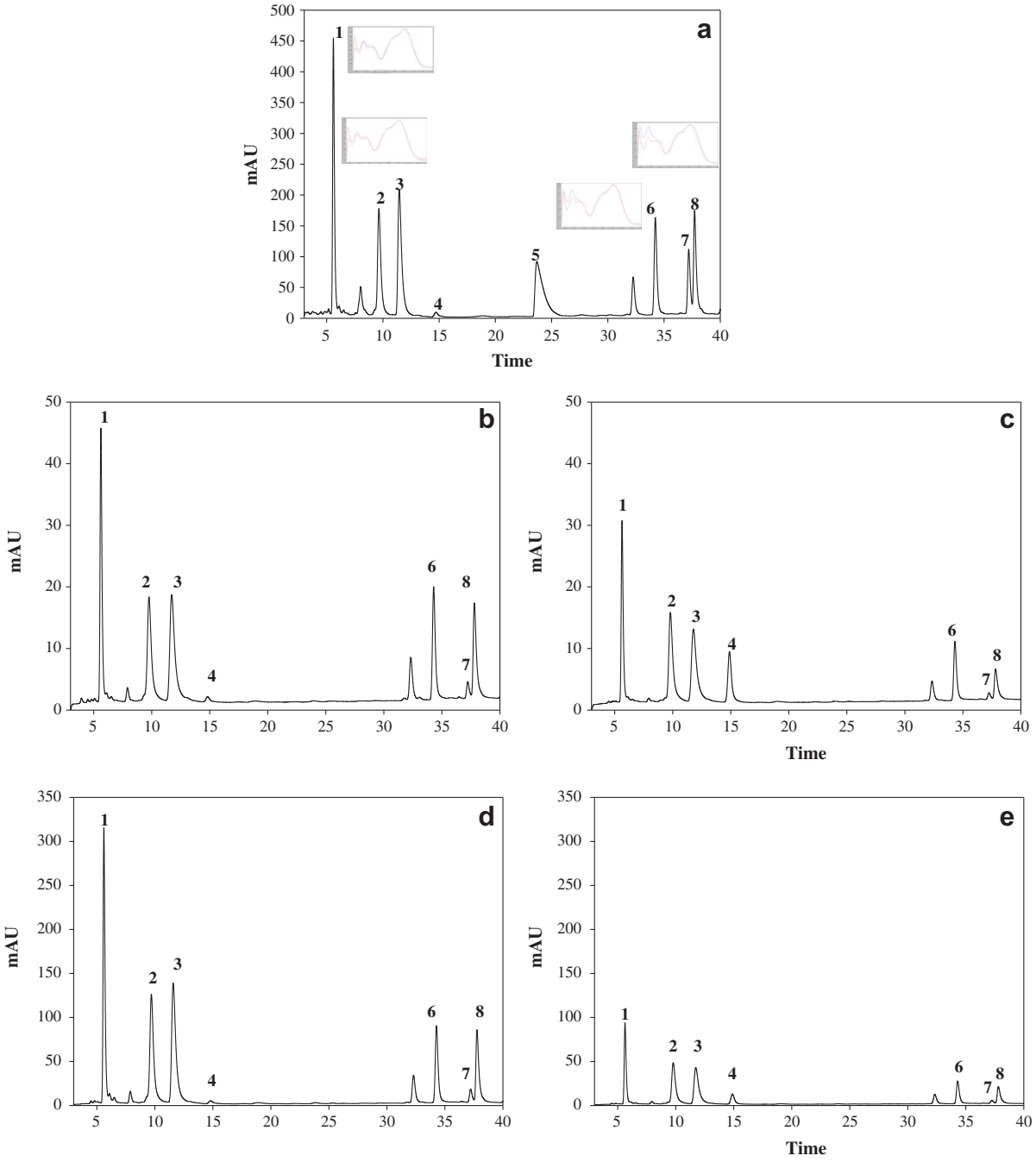

Fig. 7. HPLC chromatograms of (a) yerba mate extract at 275 nm, inserted figures correspond to DAD spectra of chlorogenic acid (red line) and non-identified peak (blue line); (b and c) CC-YM3 samples before and after of 120 days of storage at 330 nm, respectively; (d and e) CC-YM10 samples before and after of 120 days of storage at 330 nm, respectively. Peaks 3. chlorogenic acid; 4. caffeic acid; 5. caffeine and 7. rutin. Peaks 1,2,6,8 were non-identified. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds. According to Ponce Cevallos et al. (2010) and Pralhad and Rajendrakumar (2004) in the DSC assay the total or partial disappearance of thermal events (melting point) corresponding to the active compound can be considered as proof of its incorporation into the matrix.

By FT-IR analysis were identified the bands of the functional groups present in the co-crystallized powders without and with yerba mate extract (Fig. 5). All samples showed typical bands of sucrose molecule similar to those reported by Gopi et al. (2013). Signals found at 3319 cm^{-1} were assigned to the vibration (stretching) of the –OH groups. Bands attributed to the stretching of C–H groups were detected around 3012 and 2970 cm^{-1}. Symmetric and asymmetric stretching for the group $–CH_2$ were evidenced by the bands located around 2941 and 2983 cm^{-1}. Characteristic peaks for stretching C–O groups were found at 1128 and 991 cm^{-1}. The C–C stretching vibration modes were found at 1069 and 942 cm^{-1} (Gopi et al., 2013).

3.3. Physicochemical stability during storage

The hygroscopicity determination helps predict handling and packaging behavior. The water gain facilitates the occurrence of degradation reactions, which are accelerated by the effect of temperature (Schultheiss and Newman, 2009). Weight gain percentage

Table 4
Concentration of phenolic compounds and caffeine in the co-crystallized powders determined by HPLC.

Active compound	CC-YM3			CC-YM10		
	0-day	120-day	Change (%)	0-day	120-days	Change (%)
TPC[a]	0.54 ± 0.009	0.58 ± 0.01	7.4	1.72 ± 0.02	1.75 ± 0.03	1.7
Chlorogenic acid[b]	0.11 ± 0.001	0.09 ± 0.006	−19.1	0.26 ± 0.02	0.18 ± 0.07	−30.3
ChA-RC	0.14 ± 0.0003	0.11 ± 0.002	−22.2	0.31 ± 0.008	0.22 ± 0.002	−29.0
Caffeic acid[b]	0.009 ± 0.0001	0.02 ± 0.008	163.7	0.01 ± 0.003	0.04 ± 0.003	205.3
Rutin[b]	0.05 ± 0.002	0.03 ± 0.0003	−44.3	0.07 ± 0.03	0.05 ± 0.02	−29.0
Caffeine[b]	0.09 ± 0.002	0.07 ± 0.002	−16.1	0.23 ± 0.01	0.10 ± 0.11	−58.2

[a] Expressed as mg of chlorogenic acid/ g co-crystallized.
[b] mg of compound/g co-crystallized, ChA-RC: Chlorogenic acid related compounds.

of co-crystallized products stored at 75% RH and 20 °C reached the equilibrium value at around 24 h. ANOVA indicated that the concentration of the yerba mate extract and the particles size were non-significant factors ($p > 0.05$). The final moisture content attained after exposing the powder to the mentioned conditions was used to calculate the hygroscopicity (Eq. (5)). The yerba mate co-crystallized materials showed very low hygroscopicity as shown in Table 3. While, at similar conditions, yerba mate powders obtained by freeze-drying became sticky, showing a higher hygroscopicity (HG% around 15%) and poor flowability properties (Deladino et al., 2007). This drawback was previously addressed through the co-crystallization in sucrose matrix (Deladino et al., 2007) and the addition of colloidal silicon dioxide in spray-drying process (Yatsu et al., 2011).

The co-crystallized powders showed non-significant differences in the polyphenols concentration measured by Folin–Ciocalteu method during storage (Fig. 6a). Accordingly, Yatsu et al. (2011) studing the stability of a yerba mate spray-dried powder towards both, ultraviolet C radiation and temperature found that the powders were photostable for 48 h and maintained the polyphenol content at 40 °C and 75% RH for 4 months.

With regard to the antioxidant activity, on spite of the drastic conditions used in the storage assay, the maximum modifications were below 50%. Fluctuations in the inhibitory activity towards DPPH· radical of the powders were observed along storage (Fig. 6b), therefore, a linear correlation between the total polyphenols content and the DPPH· inhibition (%) could not be found. Similarly, variations in the phenolic content and the antioxidant capacity during refrigerated storage of vegetable products were reported by Piljac-Žegarac et al. (2009) and Viña and Chaves (2006) and were attributed to possible modifications in the qualitative profile of the phenolic compounds. Pinelo et al. (2004) explained that antioxidant activity fluctuations could be attributed to both the strong tendency of polyphenols to undergo polymerization reactions, whereby the resulting oligomers possess larger areas available for charge delocalization and thus, increase the antioxidant activity; and when the degree of polymerization exceeds a critical value, the increased molecular complexity and steric hindrance reduce the availability of hydroxyl groups in reaction with the DPPH radicals, which causes a resultant decrease in the antiradical capacity.

Fig. 7 shows HPLC chromatograms for free and co-crystallized yerba mate extract before and after 120 days of storage. Chlorogenic acid (t_R = 12 min), caffeic acid (t_R = 15 min), caffeine (t_R = 25 min) and rutin (t_R = 37 min) were identified by their DAD spectra and chromatographic comparisons with the standards. Also, several non-identified peaks were found (peaks 1, 2, 6 and 8) whose DAD spectra showed a high matching with that of chlorogenic acid (inserts in Fig. 7a). In the present work, we will refer them as "chlorogenic acid related compounds" and their total concentration was calculated summing the areas of peaks 1, 2, 6 and 8 and expressed as chlorogenic acid equivalents.

Table 4 shows the concentration of phenolic compounds and caffeine loaded in the co-crystallized powders before and after 120 days of storage. The changes in the amount of each compound were calculated as follows:

$$\text{Change}_{(\%)} = \left(\frac{L_{120} - L_0}{L_0}\right) \times 100 \quad (6)$$

where L_o is the active compound load before storage and L_{120} the active compound load after 120 days of storage. Values of change (%) <0 are indicative of degradation of the active compound and (%) >0 correspond to an increase in the active compound concentration during storage.

Chlorogenic acid concentration of the yerba mate powders decreased around 19% and 30% for CC-YM3 and CC-YM10, respectively. Similar behavior was observed for the chlorogenic acid related compounds (Table 4). Whereas a higher amount of caffeic acid was obtained after storage for both powders (Table 4). Chlorogenic acids are a family of esters composed of quinic acid and certain trans-cinnamic acids, most commonly caffeic, p-coumaric and ferulic acid (Jaiswal et al., 2010). On this regard, the increase in the concentration of caffeic acid could be attributed to the degradation of chlorogenic acid and related compounds under storage conditions. According to Fang and Bhandari (2011) and De Sotillo et al. (1994) some phenolic components including chlorogenic acid may be sensitive to storage conditions (light, oxygen presence, relative humidity and temperature) and may degrade into another compound. A significant reduction in the concentration of rutin and caffeine was observed, as well.

The quali-quantitative polyphenol profile determined by HPLC allowed to correlate the decrease in antioxidant activity towards DPPH· with lower values of chlorogenic acids and rutin in stored samples. The participation of caffeine and polyphenols on the overall antioxidant activity of yerba mate has been determined, previously. Anesini et al. (2012) found that chlorogenic acids presented the most potent DPPH· scavenging effect followed by caffeic acid and rutin. These results are in agreement with those found by Deladino et al. (2013) whom employed photochemiluminescence method and determined the same decreasing order for inhibition capacity. Both groups of authors found no antioxidant activity for the caffeine.

4. Conclusions

Yerba mate antioxidant powders were obtained with high entrapment yield. The co-crystallization process allowed to maintain largely the antioxidant activity of the yerba mate extract without modifications. The powders obtained showed desirable characteristics such as low water content and water activity, high solubility, low hygroscopicity and very good flowability. Co-crystallized yerba mate constitutes a promising alternative as a natural antioxidant ingredient for the formulation of functional foods.

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