Research Article

Ilex paraguariensis Pellets from a Spray-Dried Extract: Development, Characterization, and Stability

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Received 27 March 2015; accepted 26 June 2015; published online 11 July 2015

Abstract. Several studies have shown the potential use of *Ilex paraguariensis* in developing products with the aim to protect biological systems against oxidative stress-mediated damages. In the same way, technological studies have demonstrated the feasibility of obtaining dry products, by spray-drying process, from aqueous extracts of *I. paraguariensis* in laboratory. The present work was designed to develop pellets by extrusion/spheronization process, from an *I. paraguariensis* spray-dried powder. The pellets were characterized with respect to their chemical, physical, and technological properties, and the thermal and the photostability of the main polyphenol constituents were investigated. The pellets exhibited adequate size, shape, and high process yield (78.7%), as well as a good recovery of the total polyphenols (>95%) and a good dissolution in water (89.44 to 100.05%). The polyphenols were stable against light when conditioned in amber glass bottles; unstable against heat when the samples were conditioned either in open glass bottles or in hermetically sealed glass bottles and demonstrated to be hygroscopic and sensible to the temperature, especially when stored in permeable flasks. These findings pointed to the relevance of reducing the residual moisture content of pellets as well as of conditioning them in opaque humidity tight packages under low temperatures. The feasibility of obtaining pellets from an *I. paraguariensis* spray-dried powder using extrusion/spheronization technique was, for the first time, demonstrated. This finding represents a novelty for the herbal products in both pharmaceutical and food fields.

KEY WORDS: extrusion/spheronization; *Ilex paraguariensis*; pellets; spray-dried powder.

INTRODUCTION

Ilex paraguariensis is widely consumed in South America and it is gaining rapid introduction into the world market, either as a nonalcoholic beverage (tea and infusion) or as an ingredient of foods and dietary supplements. It has been shown that *I. paraguariensis* has a high antioxidant activity (1–4). It is hypocholesterolemic (5,6), hepatoprotective (7), diuretic (8), and beneficial to the cardiovascular system (5). It is also able to protect DNA (9,10) and low-density lipoproteins from oxidation (11).

In the same way, several studies have shown the potential use of *I. paraguariensis* in developing products with the aim to protect biological systems against oxidative stress-mediated damages (12–14). Technological studies have demonstrated, for example, the feasibility of obtaining dry products, by spray-drying process, from aqueous extracts of *I. paraguariensis* in laboratory (15–17).

The processing of plant extractive solutions in dry extracts leads to products with higher concentration and stability and more homogeneous distribution of chemical constituents, as well as with easier transport and storage. However, dry extracts in general exhibit technological limitations, such as fine particles presenting low density and poor flow, which impair the direct production of pharmaceutical dosage forms like capsules or tablets, requiring their transformation into intermediate products, such as granules (18).

The granulation process of products containing high levels of dry extracts remains a difficult problem to solve, especially in the case of products obtained by spray drying using colloidal silicon dioxide as excipient. The low density and size of these products have been the major limitation for their granulation, even with the use of techniques such as fluidized bed (19). The dry granulation, even if viable, has resulted in rough products presenting porous and poor flow (19–21). Alternatively, pellets obtained by the extrusion/spheronization process are drug delivery systems that offer technological advantages, for example,

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better flow properties, less friable dosage forms, uniform size distribution, ease of coating, and uniform packing (22).

In this context, the present work was designed to develop pellets by extrusion/spheronization process, from an *I. paraguariensis* spray-dried powder (SDP) and characterize their chemical, physical, and technological properties, as well as to investigate the thermal and the photostability of the main polyphenol constituents present in pellets.

MATERIALS AND METHODS

Chemicals

Microcrystalline cellulose (MCC; Avicel® PH 101, Blanver, São Paulo, Brazil) and colloidal silicon dioxide (Aerosil® 200, Degussa, São Paulo, Brazil) were used as excipients. Chlorogenic acid and rutin hydrate (Sigma-Aldrich, Steinheim, Germany) were used as external standards. Liquid chromatography-grade methanol (Tedia, Fairfield, USA), acetic acid (Cromoline, São Paulo, Brazil), and purified water (Milli-Q system, Millipore, Bedford, MA, USA) were used for mobile phase preparation.

Plant Material

I. paraguariensis leaves and stems were supplied by the Instituto Brasileiro do Meio Ambiente—IBAMA (Ilópolis, RS, Brazil). The specimen was identified and deposited at the Herbarium of the Universidade Federal do Rio Grande do Sul (142488). The traditional method of “erva-mate” production was employed; briefly, the raw material was stabilized by roasting, and afterwards, it was dried and ground.

Spray-Dried Powder Preparation

An aqueous extractive solution (ES) was prepared by decoction of *I. paraguariensis* leaves at 96°C for 15 min, at a plant:solvent ratio of 1.5:10. The ES was cooled down to 40°C and filtered. Colloidal silicon dioxide was added to ES at a 3:7 ratio (excipient:dry residue) and mixed for 30 min. The resulting dispersion was spray dried using a Niro Production Minor atomizer (GEA, Copenhagen, Denmark) under the following operating conditions: 10,900 rpm disk rotation rate, 177°C inlet air temperature, 99°C outlet air temperature, and 143 mL/min feed flow (17). The SDP presented a moisture content of 6.64%.

Pellets Preparation

Pellets formulation was composed by SDP (40%, w/w) and MCC (60%, w/w). Thus, 400 g of *I. paraguariensis* SDP and 600 g of MCC were blended for 5 min in a planetary mixer and, then, 700 mL of water was slowly added to the mixture under constant stirring for 15 min. The wet mass was extruded at room temperature (18–22°C) through a die of 1 mm in diameter at 16 rpm (Caleva 20 extruder, Dorset, UK). The extrudate was spheronized (Caleva 250, Dorset, UK) for 4 min on a 22.5-mm diameter radial cut plate rotating at 1000 rpm. The pellets were dried at 40°C using a fluid bed dryer (Mycrolab Hüttlin GmbH, Steinheim, Germany) until

the weight loss on drying of the pellets reached 3.64% (30 min).

Pellets Characterization

Process Yield and Moisture Content

The process yield was calculated as the difference between the theoretical weight of the formulation and the weight of pellets obtained at the end of the granulation process considering the particle size between 0.80 and 1.18 mm. The moisture content was determined by titrimetric method (23).

Particle Size Analysis

The size distribution was determined using mechanical sieving (Haver and Bocker, Westfalen, Germany) containing a set of sieves: 0.71, 0.80, 0.9, 1.0, 1.12, 1.18, and 1.25 mm. Samples of 10 g were shaken for 2 min with an interval of 10 s and amplitude of 1. The mean diameter was determined from a cumulative percentage undersize graph.

Scanning Electron Microscopy

Photomicrographs were taken using a JEOL JSM 6060 microscope (Tokyo, Japan), at a voltage of 10 or 20 kV. Intact and cryo-fractured pellets were previously mounted on aluminum stubs using double-sided adhesive tape and vacuum coated with a thin layer of gold. Samples were frozen by immersion, at ambient pressure for 60 s in liquid nitrogen to below –160°C, and fractured with a hammer.

Density

The density was determined using helium pycnometer (Ultrapycnometer 1000, Quantachrome Instruments, Boynton Beach, USA) by measuring the pressure difference when a known quantity of helium under pressure was allowed to flow from a precisely known reference volume into a sample cell containing pellets (3 g).

Specific Surface Area and Pore Size Distribution/Volume

Pellets (0.80–1.20 mm size fraction) were dried in oven for 12 h. Afterwards, the samples were outgassed by the Autosorb-1 equipment (BET method, Quantachrome Instruments, Boynton Beach, USA) at 70°C for 180 min. After cooling the tubes, the surface area was analyzed by the same instrument. Nitrogen was added in known quantities into the evacuated tube containing the sample. A gradual increase in the quantity of nitrogen gas increased the pressure in the sample tube. When the pressure in the tube had equilibrated following each introduction of nitrogen gas, the pressure was recorded. The pressure data were used, in turn, to calculate the volume of gas adsorbed. The volume of gas adsorbed was measured as a function of relative pressure. Relative pressure was the ratio between the pressure in the sample tube and the saturation vapor pressure of the adsorbate gas (*i.e.*, the pressure at which the adsorbate gas liquefies). The saturation pressure was measured for every sample tube pressure data

point. The surface area and pore size/volume parameters were calculated from the isotherm data.

Hausner's Ratio and Carr's Index

The density parameters of the SDP were determined using 10.0 g of sample in a 50-mL graduated cylinder mounted on a mechanical tapping device (24) (J. Engelsmann AG, Ludwigshafen, Rhein, Germany). The bulk density was calculated as the ratio between the sample weight (g) and the initial volume (mL), and the tapped density as the ratio between the sample weight (g) and the final volume (mL). The Carr's index (25) and the Hausner's ratio (26) were calculated according to the following equations:

$$\text{Carr's index (\%)} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Tapped density}} \times 100$$

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of Repose

The angle of repose (23) of the SDP was measured according to the following equation:

$$\text{Tg } \theta = h/r$$

where Tg θ is the tangent of the angle of repose, h is the height, and r is the radius.

Liquid Chromatography Analysis

Chromatographic Conditions

The liquid chromatography (LC) analysis was performed as described by Silva and cols. (27), using a Shimadzu Prominence equipment (Kyoto, Japan) coupled to a SPD-20A UV-Vis detector. The stationary phase was a Shimadzu RP-18 column (CLC-ODS (M) 250×4.6 mm i.d., 5 μ m particle size) guarded by a Waters precolumn (20×3.9 mm i.d., 10 μ m particle size). The mobile phase consisted of (A) acetic acid 2.0% (v/v) and (B) methanol:water (85:15, w/w). The gradient elution was 31% B (0–10 min), 31–56% B (10–25 min), 56% B (25–33 min), 56–77% B (33–45 min), 77–56% B (45–50 min), and 56–31% B until 60 min. The flow rate was 0.7 mL/min and the injection volume was 20 μ L. The detection wavelength was 340 nm, and the analysis was carried out at room temperature.

Standard Curves

Chlorogenic acid (CA) and rutin (RU) were used as external standards. They were dissolved in methanol:water (50:50, v/v) and diluted to obtain the concentrations of 2.0, 4.0, 6.0, 8.0, and 10.0 μ g/mL. These solutions were filtered through a 0.45- μ m membrane filter (Millipore, HVLP). The linear equations ($n=5$) were $y=67,255x-13,006$ ($r^2=0.9993$)

and $y=43,584x-7053.5$ ($r^2=0.9991$) for CA and RU, respectively.

Sample Preparations

Pellets (136.5 mg) were dispersed in 50.0 mL of water. From this colloidal dispersion, an aliquot of 2.0 mL was diluted to 10.0 mL with a mixture of methanol:water (50:50, v/v). The resulting solution was filtered through a 0.45-mm membrane (Millipore, HVLP) and analyzed by LC, taking into account pellet residual moisture determined by titrimetric method (23). For the stability tests, the moisture content was determined before the LC analysis for samples collected in each time. The content of neo-chlorogenic acid, cryptochlorogenic acid, P4, P5, and P6 in the samples was determined using CA calibration curve, since they exhibit UV spectra pattern of caffeoylquinic acid derivatives with UV max absorption bands at 328 nm (27). The content of RU was calculated by the corresponding equation. Total polyphenol content in the samples was obtained by the sum of the content of neo-chlorogenic acid, chlorogenic acid, cryptochlorogenic acid, P4, P5, and P6.

Method Validation

The LC method was validated for specificity, linearity, precision (repeatability and intermediary precision), accuracy, and detection and quantitation limits (28). The specificity was evaluated by analyzing blank pellets containing only the excipients (colloidal silicon dioxide and microcrystalline cellulose), and the polyphenols were identified based on their UV spectra between 200 and 400 nm and their retention times. The accuracy of the method was evaluated by spiking known amounts of CA and RU standards at three different concentration levels (1.0, 5.0, and 10.0 μ g/mL) into blank pellet solution (methanol:water, 50%, v/v). At each level, samples were prepared in triplicate and analyzed by LC. The accuracy in the presence of the matrix was determined by subtracting the measured amount of polyphenols from the spiked amount, divided by spiked amount, and multiplied by 100.

The method was specific (data not shown) and the regression coefficients were $r^2=0.9998$ for CA and $r^2=0.9999$ for RU standard curves. When the standard was spiked into blank pellet solution, the regression coefficient was similar. The relative standard deviations (R.S.D.) of the slope from the three calibration curves were 0.33 and 0.21%, respectively, for CA and RU solutions (methanol:water, 50%, v/v) and 0.63 and 0.45%, respectively, for CA and RU into spiked blank pellet solution (2.73 mg/mL, aqueous solution). The curve equations were, respectively, $y=80,789.9x-13,046.6$, $y=44,240.1x-2887.5$, $y=868.2x-93.74.2$, and $y=193.6x-1241.9$, where y is the peak area and x is the concentration of the external standards. The observed repeatability (R.S.D.=0.43% for CA; R.S.D.=3.20% for RU) and the intermediate precision (R.S.D.=0.50% for CA; R.S.D.=1.36% for RU) were adequate. The recovery of CA (96.8 to 102.2%) and RU (99.3 to 100.5%) from blank pellet solution showed that the method was accurate and that the pellet excipients did not influence the analysis. The LOD and LOQ of CA (0.157 and 0.162 μ g/mL, respectively) and RU (0.093 and 0.094 μ g/mL, respectively) showed the excellent sensitivity of the method.

Dissolution Test

The *in vitro* dissolution test of pellets presenting size range between 0.80 and 1.18 mm was carried out according to the USP 31 apparatus II procedure (Pharmatest PTWS II, Hainburg, Germany), using a paddle speed of 75 rpm and 700 mL of dissolution media at $37 \pm 0.5^\circ\text{C}$ ($n=6$) (23). The following dissolution media were employed: purified water (pH 5.5), HCl 0.1 N (pH 1.2), or phosphate buffer (pH 6.8). Aliquots (5 mL) of the dissolution media were manually taken after 5, 15, 30, 45, and 60 min, filtered through a 0.45- μm membrane (Millipore, HVLP), and analyzed using the above-described LC method.

Stability

Photostability

The photostability test was carried out according to the ICH (29). Pellets were exposed to UVC radiation (Light express LE UV, 254 nm, 30 W) at three different storage conditions: amber glass bottles, transparent glass bottles, or open dishes. The lamp was fixed to a chamber ($100 \times 16 \times 16$ cm) in a horizontal position, at a distance of approximately 10 cm. The chamber was internally coated with mirrors, in order to distribute the radiation uniformly. A sample wrapped in aluminum foil was used as a dark control to evaluate the influence of temperature into the chamber (approximately 27°C) on pellet stability. The polyphenol content in the samples was measured in triplicate 0, 12, 24, and 48 h after irradiation using the above-described LC method.

Thermal Stability Test

The thermal stability test was carried out at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for 6 months to evaluate the stability of the polyphenols under accelerated conditions (30,31). The pellets were conditioned in transparent glass bottles or polyethylene ethyl (PET) bottles (semipermeable material). The polyphenol content in the samples was measured in triplicate at 0, 1, 2, 3, 4, 5, and 6 months after exposition in a climatic chamber (Nova Ética, SP, Brazil) using the above-described LC method. This condition was chosen to evaluate the effect of temperature and relative humidity on the stability of the polyphenols.

Statistical Analysis

The results were analyzed either by Student's test or by the analysis of variance (ANOVA) followed by Tukey's test for significance at p values less than 0.05.

RESULTS

Physical, Chemical, and Technological Characterization of the Pellets

Table I and Fig. 1 show the main technological characteristics of the pellets, as process yield (78.7%), residual moisture (3.64%), and spherical shape.

Table I. Technological Characterization of Pellets Containing 40% of Spray-Dried Powder and 60% of Microcrystalline Cellulose

Technological parameters	
Yield (%)	78.7
Moisture content (%)	3.64
Mean diameter (mm)	1.10
Apparent density (mg/mL)	1.60
Specific surface area (m^2/g)	6.92
Pore size ^a (Å)	2.95
Pore volume ($10^{-3} \text{ cm}^3/\text{g}$)	10.21
Hausner's ratio	1.00
Carr's index (%)	0.34
Angle of repose	26

^a Total pore volume for pores with radius lower than 188.40 Å at $P/P_0=0.946298$

The particle size distribution of a powder is a parameter which expresses the relative amount (%) of particles distributed within a particle size range. It was determined by analyzing the particle size of the pellets using different nominal mesh sieves. The percentages of retained particles were 20.3% (1.25 mm), 18.6% (1.18 mm), 21.2% (1.12 mm), 24.4% (1.0 mm), 12.4% (0.9 mm), 2.1% (0.8 mm), 0.8% (0.71 mm), and 0.2% (pan). Therefore, the pellets were produced within the range of 0.80 and 1.18 mm (mean diameter of 1.10 mm).

The polyphenol LC profile of pellets is presented in Fig. 2. The presence of peaks with the retention time (Rt) of the four polyphenols previously identified by Silva and cols. (27) is observed, neo-chlorogenic acid—NCA (Rt 6.97 min), chlorogenic acid—CA (Rt 10.84 min), crypto-chlorogenic acid—CCA (Rt 11.62 min), and rutin—RU (Rt 30.36 min). The non-identified peaks referred by Silva and cols. (27), P4 (Rt 27.02 min), P5 (Rt 27.68 min), and P6 (Rt 30.85 min), corresponding probably to isomeric dicaffeoyl esters of quinic acid (*i.e.*, 3,4-*O*-dicaffeoyl, 4,5-*O*-dicaffeoyl, 3,5-*O*-dicaffeoyl, or 1,5-*O*-dicaffeoyl esters), could be also observed. Table II shows the polyphenol content of the SDP and the corresponding recovery in pellets.

Dissolution Test

The release profiles of pellets in different media are presented in Fig. 3. In water (pH 5.5), the maximum dissolution of the polyphenols, individually, was in the range of 89.44 and 100.05%, where only RU reached 100% (Fig. 3a). In acid (pH 1.2) (Fig. 3b) and buffer medium (pH 6.8), all the polyphenols presented low release (Fig. 3c).

Photostability

The polyphenol content of pellets after exposition to UVC radiation is shown in Fig. 4. After 48 h of exposure, no significant change was detected in the LC profile. The polyphenols were stable against light when conditioned in amber glass and control bottles; however, after 48 h of exposure, the polyphenol content of pellets was reduced ($p < 0.05$) when the samples were stored in open dishes or in transparent glass bottles. In open dishes, NCA, CA, CCA, P4, P5, and P6 contents showed significant reduction over the time of the

Fig. 1. Photomicrograph of pellets. **a** Pellets with $\times 27$ magnification; **b** pellets with $\times 75$ magnification; **c** cryo-fracturated pellets with $\times 90$ magnification; **d** pellets after dissolution test with $\times 85$ magnification

experiment. In transparent glass bottles, only NCA, CA, CCA, and P6 were unstable after 48 h of exposure to light. The moisture content of all samples presented no significant difference ($p < 0.05$) during the photostability test (data not shown).

Accelerated Testing

The results of pellets accelerated testing are presented in Figs. 5 and 6. Pellets showed to be hygroscopic, increasing their residual humidity when conditioned in PET bottles. After 6 months, the moisture content of the

samples stored in PET bottles increased from 3.64 to 6.83% and their color became brown. Pellet samples stored in transparent glass bottles presented no significant difference ($p < 0.05$) in the moisture content over the accelerated testing (data not shown), but their color became brown too. When the pellets were stored in transparent glass bottles (impermeable material), all polyphenols evaluated presented a significant decrease ($p < 0.05$) in their content. After 6 months, the percentages of degradation were 33.5% (NCA), 29.5% (CA), 27.4% (CCA), 40.1% (P4), 39.1% (P5), 19.5% (RU), and 34.2% (P6). The total polyphenol content of pellets decreased from 199.4 to

Fig. 2. Chromatographic profile of pellets. NCA neo-chlorogenic acid; CA chlorogenic acid; CCA crypto-chlorogenic acid; RU rutin; P4, P5, and P6 isomeric dicaffeoyl esters of quinic acid

Table II. Polyphenol Content of Spray-Dried Powder (SDP) and Pellets ($n=3$)

Peaks	SDP (mg/g) ($\bar{X}$; S.D.)	Pellets (mg/g) ($\bar{X}$; S.D.)	Recovery (%)
NCA ^a	49.74 (0.02)	47.97 (0.12)	96.4
CA	43.63 (0.29)	43.41 (0.01)	99.5
CCA ^a	41.52 (0.02)	41.39 (0.67)	99.7
P4 ^a	20.05 (0.36)	19.21 (0.07)	95.8
P5 ^a	16.10 (0.52)	14.91 (0.33)	92.6
RU	15.85 (0.45)	13.14 (0.23)	82.9
P6 ^a	22.62 (0.08)	19.37 (0.33)	85.6
Total ^b	209.52 (1.50)	199.40 (0.85)	95.2

NCA neo-chlorogenic acid, CA chlorogenic acid, CCA crypto-chlorogenic acid, RU rutin, SDP spray-dried powder, S.D. standard deviation

^a Calculated by the chlorogenic acid linear equation

^b Sum of neo-chlorogenic acid, chlorogenic acid, crypto-chlorogenic acid, P4, P5, and P6 concentrations

136.5 mg/g. In the same way, when the pellets were stored in PET bottles (semipermeable material), all polyphenols evaluated presented a significant decrease ($p<0.05$) in their content; after 6 months, the percentages of degradation were 55.5% (NCA), 33.32% (CA), 20.2% (CCA), 51.8% (P4), 61.4% (P5), 67.4% (RU), and 37.3% (P6). The total polyphenol content of pellets decrease from 199.40 to 114.8 mg/g.

DISCUSSION

Pellet Physical Characterization and Polyphenol Content

The extrusion and spheronization process of the SDP required about 70% of water (percentage of the total weight of solid raw materials) in order to obtain a wet mass with a suitable consistency to extrudate. During the spheronization step, in order to avoid the agglomeration of the spheroidal granules, 0.5% of MCC was sprinkled on the spheroidal granules while they were rotating in the spheronizer.

Taking into account the total weight of the wet mass, the percentage of water necessary to obtain a wet mass with a suitable consistency was about 41%, which is in agreement with a previous study of Lustig-Gustafsson and cols. (32). The authors investigated the influence of the water content of the wet mass on the formation and properties of pellets composed by eight non-related drugs of different solubility (boric acid, glycine, glucose, ascorbic acid, caffeine, two batches of lactose monohydrate, paracetamol, and 5-aminosalicylic acid) and verified that the optimum water content of the wet mass necessary to form round pellets with a narrow particle size distribution was in the range of 30 to 40.6% and is a function of the solubility of the model drugs when incorporated into equal parts of MCC.

Pelletization seemed to occur by the mechanism described by Rowe (33), because some granules presented a scar right in the center, which can be considered a transitional form between the dumbbell and the spheroid shape. According to this author, the extrudate broke on the friction plate of the spheronizer into small cylinders which went through several

Fig. 3. Pellets dissolution profile ($n=6$). **a** Release profile in water (pH 5.5); **b** release profile in HCl 0.1 N (pH 1.2); **c** release profile in buffer phosphate (pH 6.8). NCA neo-chlorogenic acid; CA chlorogenic acid; CCA crypto-chlorogenic acid; RU rutin; P4, P5, and P6 isomeric dicaffeoyl esters of quinic acid

Fig. 4. Polyphenol content of pellets after 48 h of exposure to UVC radiation ($n=3$). *NCA* neo-chlorogenic acid; *CA* chlorogenic acid; *CCA* crypto-chlorogenic acid; *RU* rutin; *P4*, *P5*, and *P6* isomeric dicaffeoyl esters of quinic acid; significant statistical differences were determined by Tukey's test ($p<0.05$); differences between the concentrations over time (h) were represented by letters (a , b)

shape changes, *i.e.*, cylinders with rounded ends, dumbbells, ellipsoids, and finally spheroids, which could be evidenced by the photomicrographs (Fig. 1).

Despite the addition of MCC to avoid pellet agglomeration in the spheronization process, the pellets

presented a mean diameter of 1.1 mm, high yield (78.7%), and residual moisture content (3.6%), characteristics which become this product appropriated for obtention of derivative forms, as tablets or capsules, or as final product (sachets).

Fig. 5. Total polyphenol content of pellets under accelerated condition (40°C/75% relative humidity/6 months) ($n=3$). *Glass* stored in transparent glass bottles; *PET* stored in polyethylene ethyl bottles; significant statistical differences were determined by Tukey's test ($p<0.05$); differences between glass and PET bottles were represented by an asterisk, while differences over time (months) were represented by letters (a , b , c , d)

Fig. 6. Polyphenol content of pellets under accelerated condition ($40^{\circ}\text{C}/75\%$ relative humidity/ 6 months) ($n=3$). *NCA* neo-chlorogenic acid; *CA* chlorogenic acid; *CCA* crypto-chlorogenic acid; *RU* rutin; *P4*, *P5*, and *P6* isomeric dicaffeoyl esters of quinic acid; *Glass* stored in transparent glass bottles; *PET* stored in polyethylene ethyl bottles; significant statistical differences were determined by Tukey's test ($p<0.05$); differences between glass and PET bottles were represented by an asterisk, while differences over time (months) were represented by letters (a, b, c, d)

Three commonly reported methods for testing the pellet flow properties were applied: the Carr's index (CI), the Hausner's ratio (HR), and the angle of repose (23). A CI below 15, an HR lower than 1.25, and an angle of repose lower than 30° characterize free-flowing materials. Based on the results, the pellets could be classified as a material with good flow properties.

The polyphenol content in SDP was compared to that found in the corresponding pellets, taking into account the moisture content of both. Individually, the polyphenol present in the pellets were in a range of 82.9 to 99.7% that present in SDP. The total polyphenol (sum of neo-chlorogenic acid, chlorogenic acid, crypto-chlorogenic acid, P4, P5, and P6 contents) in the pellets reached a value higher than 95% that contained in SDP, what can be considered an excellent result

considering the lability of this class of compounds and the complex herbal matrix.

Dissolution Test

After the dissolution test, pellets remained intact and separated without swelling. It is well known that MCC, when extruded and spheronized, loses its swelling properties. In this way, pellets will only disintegrate if the amount of drug is larger than the MCC capacity of keeping the pellet structure. In MCC pellets with higher amount of excipient, the water-soluble drug is washed out of pellets leaving pores in the structure (34). This could be seen in the photomicrographs presented in Fig. 1d, where pores were found in the pellets after the dissolution test. In this case, the MCC would act as a

matrix/reservoir and the extract would be released by diffusion.

In acid medium (pH 1.2), all the polyphenols presented low release (Fig. 3b), as we could expect for compounds with weak acid characteristics. Unexpectedly, a similar behavior was also observed in a buffer medium (pH 6.8) (Fig. 3c), denoting that other factors were involved. Since sink condition has been respected for all dissolution media (CA solubility = 25 mg/mL at 25°C in water; RU solubility = 0.12 mg/mL at 25°C in water), we checked the stability of the polyphenols into dissolution media (pH 1.2 and 6.8). No degradation of polyphenols was observed in both media since the samples remained stable for 3 days at 24°C (data not shown). We are looking for an explanation for this phenomenon, being the effect of the salts from the buffer solution or lack of release from the MCC matrix in this pH cannot ruled out.

Photostability

After 48 h of exposition to UVC radiation, the polyphenol content of pellets was reduced ($p < 0.05$) when the samples were stored in open dishes or in transparent glass bottles. However, in a previous report (17), we demonstrated that the polyphenols present in SDP raw material showed to be stable against light over the time under the same conditions employed in the present study. In that study, we suggested that the protective effect was provided by the use of colloidal silicon dioxide as excipient because of its ability to reflect/scatter UV and visible radiations (35). Considering this, the sensibility of the polyphenols from pellets could be related to the new structure of the matrix produced by the addition of MCC, granulation with water, extrusion, and spheronization.

Accelerated Testing of Stability

In order to evaluate the influence of the temperature and relative humidity on the polyphenol stability, two different types of packaging material were tested: transparent glass bottles (impermeable material) and PET bottles (semipermeable material). After 6 months, the content of all polyphenols evaluated presented a significant decrease ($p < 0.05$) in both conditions (transparent glass and PET bottles). However, the reduction of polyphenol content was more prominent in the pellets stored in PET bottles, especially for NCA, P4, P5, and RU, which presented a degradation superior to 50% of the initial concentration.

These results were very similar to that obtained in a previous study (17) where the stability of SDP was evaluated under the same conditions. According to that study, after 4 months, the samples conditioned in PET bottles presented an increase in the residual moisture content (6.64 to 13.96%) and the polyphenols of the SDP were more degraded than that samples stored in impermeable glass bottles. According to the authors, the degradation of polyphenols in SDP could be associated to the residual moisture content, since the degradation was more pronounced in the samples which presented high residual moisture content. The presence of water could facilitate the occurrence of the residual activity of the peroxidases, a very thermostable enzyme present in the *I. paraguariensis* raw material (36,37,38).

The CCA concentration showed an anomalous increase in the first months, when pellets were stored in both transparent glass and PET bottles. After that, CCA concentration decreased significantly ($p < 0.05$), until the sixth month, to a concentration lower than the initial. This phenomenon was also observed in our previous study (17).

CONCLUSION

The feasibility of pellets from an *I. paraguariensis* spray-dried powder using extrusion/spheronization technique is being demonstrated opening possibilities for new products from this traditional raw material in both, pharmaceutical and food fields. The pellets exhibited adequate size, spherical shape, and very good yield. When stored in amber in tight amber glass bottles, the total polyphenol were stable against the exposure to UVC radiation for 48 h. In contrast, the polyphenols demonstrated to be sensible to the temperature, especially when the residual moisture is high what indicate the need of conditioning it in opaque and humidity tight packing under low temperatures.

ACKNOWLEDGMENTS

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the financial support and the scholarships.

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