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ORIGINAL ARTICLE

Assessment of stability of a spray dried extract from the medicinal plant *Bidens pilosa* L.

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Abstract Spray drying has been successfully employed for the encapsulation of herbal bioactive compounds resulting in stable phytopharmaceutical preparations. *Bidens pilosa* L. is a South American medicinal plant with proved antimalaric, hepatoprotector and antioxidant activities, generally linked to their secondary metabolites, flavonoids and polyacetylenes. In this work the physicochemical stability of an optimized spray dried composition from a *B. pilosa* extract was evaluated at three different stress storage conditions in open containers and in sealed sachets. High performance liquid chromatography was employed to monitor the concentration of three marker compounds over 12 months. Color variation of the stored samples was evaluated by using a color spectrophotometer. It was observed that the concentration of the monitored compounds of the plant decreases more drastically in samples stored in open containers. The two flavonoids monitored, rutin and hyperoside, showed lower degradation than the polyacetylene. The concentration of the markers did not change significantly at the lowest temperature. With regard to color, darker hues were observed at higher temperatures and storage times. This study showed that the storage conditions cause significant impact on stability of standardized spray dried *B. pilosa* extract.

1. Introduction

Several studies have proved the beneficial properties of herbal medicinal products (HMP) for human health. Different kinds of molecules have been isolated and their pharmacological and physicochemical properties have been studied (Bero et al., 2009; Eldridge et al., 2002; Harbone and Williams, 2000; Li and Vederas, 2009; Pérez-Jiménez et al., 2010). However, those compounds and extracts need to be properly formulated in order to achieve their physiological target and exert the expected pharmacological activity. Factors such as low permeability and/or solubility could affect the delivery and absorption of bioactive compounds (Fang and Bhandari, 2011). On the other hand the shelf-life of HMP should be evaluated in order to guarantee the stability during the period of use. Degradation reactions promoted by temperature, pH, humidity, oxygen and light must be controlled. Stress storage conditions are employed to predict product shelf-life through accelerated degradation reactions (Bott et al., 2010). HMP are complex mixtures of different classes of chemical compounds, such as carbohydrates, lipids, proteins, and secondary metabolites among others.

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In general, liquid HMP present lower physicochemical and microbiological stability than the corresponding dried forms, justifying the development of drying and encapsulation processes in order to improve the quality and safety of those phytopharmaceutical preparations (Ameri and Maa, 2006; Bakowska-Barczak and Kolodziejczyk, 2011; Bhandari and Howes, 1999; Bott et al., 2010; Jiménez-Aguilar et al., 2011; Rocha et al., 2011; Yatsu et al., 2011). The encapsulation of bioactive compounds from vegetal samples has been employed to solve problems related to volatile losses, premature instability and low bioavailability. This technique also improves product handling and storage. There are different encapsulation methods which could be divided in physical (spray drying and spouted bed drying), chemical (interphase polymerization) and physicochemical (conservation and phase separation) (Braga and Oliveira, 2007; Desai and Park, 2007; Oliveira and Petrovick, 2010; Sollohub and Cal, 2010).

Spray drying is one of the most commonly employed techniques for drying and encapsulation since drying occurs rapidly (low residence time) and the core reaches temperatures generally lower than 100 °C, therefore the thermal degradation of the product is reduced (Ameri and Maa, 2006; Fang and Bhandari, 2011; Yatsu et al., 2011).

Bidens pilosa L. (Asteraceae) is a South American medicinal plant nowadays disseminated all over the world, mainly in tropical and subtropical regions. Biological activities of the plant such as antimalaric, hepatoprotector, antimicrobial and antioxidant have been linked to its flavonoids and polyacetylenes (Andrade-Neto et al., 2004; Krishnaiah et al., 2011; Kumari et al., 2009; Yuan et al., 2008). It is important to remark that polyacetylenes which are common in the *Bidens* species are very active but also very unstable molecules when isolated (Brandão et al., 1997).

This work aims to evaluate the physicochemical stability of a standardized spray dried extract of *B. pilosa* stored at different temperatures and relative humidities, monitoring the concentration of bioactive compounds, moisture uptake and color variation.

2. Material and methods

2.1. Plant material

Individuals of *B. pilosa*, Asteraceae were collected at the Experimental Farm of IAC (Agronomic Institute of Campinas, Monte Alegre do Sul, SP, Brazil) during the summer season. Vegetal samples were authenticated by Prof. Dr. M. Groppo and a voucher specimen (collection No. SPFR 12751) was deposited in the herbarium of the Biology Department of the University of São Paulo, Campus Ribeirão Preto, SP, Brazil. The aerial parts of the plant were dried in an air circulation oven at 37 °C and milled in a knife-mill (model MA-680, Marconi, Piracicaba, SP, Brazil), until a mean diameter of 0.3 mm.

2.2. Extraction of bioactive compounds

The extraction conditions of bioactive compounds of *B. pilosa* were previously studied and optimized by our group using response surface methodology (Cortés-Rojas et al., 2011). Briefly, extract was prepared in a stainless steel extractor coupled to a thermostatic water bath set at 66.2 °C using ethanol 62.7% (v/v) and a plant-to-solvent ratio of 1:10 (w/v). Extraction mixture was maintained under agitation for 30 min by using a radial impeller. Afterward, the extract was filtered through filter paper (80 g/m^2, 14-mm pore diameter, and thickness of 205 mm; J. Prolab, S. José dos Pinhais, PR, Brazil) using a Buchner funnel (No. 4, diameter of 150 mm) connected to a vacuum pump (model 131, Prismatec, São Paulo, Brazil) set at 500 mmHg. The filtered extract was concentrated in a rotary evaporator (Fisatom-802, Fisatom, São Paulo, SP, Brazil) at a vacuum pressure of 700 mmHg and a temperature of 40 °C until a dry residue of 8.4% w/w. was attained

2.3. Spray drying

The spray drying conditions were previously optimized by our group, briefly consisting of an inlet gas temperature of 155 °C, air flow rate of 1.67×10^{-2} m^3/s, extract atomization (feed flow rate) of 1.49×10^{-4} kg/s and a mixture of microcrystalline cellulose MC102 and Aerosil® (37:13) as drying carriers. Drying was carried out in a bench-top Lab-Plant SD-05 spray dryer (Lab-Plant UK Ltd., Huddersfield, UK) with a concurrent flow regime controlled inlet drying gas temperature and monitoring outlet gas temperature. The herbal extract preparations were fed into the dryer by a peristaltic pump connected to a two-fluid atomizer, with an internal orifice of 0.7 mm.

2.4. Stability test

The stability testing of the spray dried phytopharmaceutical compositions of *B. pilosa* was carried out under the storage conditions presented in Table 1. Relative humidity and temperature were controlled by climatic chambers Nova ética®, B.O.D 411D and Nova ética® 420E (Vargem Grande Paulista, Brazil) and a refrigerator Electrolux Air flow system DC38. Approximately 1 g of dried product was placed in hermetic PVC–Aluminum sachets or glass containers (2 cm diameter and 4 cm height).

2.5. Chromatographic analysis

The chromatographic profile of the *B. pilosa* dried extracts was performed in a Shimatzu LC-20A series and a LC-6A double pump (Shimatzu Corporation, Kyoto, Japan) using a C-18 column (Shimatzu Shim-Pack CLC(M) 4.6 mm × 25 cm, 5 μm, 100 Å) at 30 °C. Chromatograms were recorded at 254 nm. Gradient acetonitrile–acidified water at pH 2.8 was used as mobile phase. Acetonitrile concentration was gradually increased as follows: 0–5 min, 10%; 5–7 min, 20%; 7–31 min, 31%; 32–44 min, 40%; 44–50 min, 100%; and 55–58 min, 10%.

Samples were exactly weighed in an analytic balance (Mettler Toledo AG204, Switzerland) and diluted at a concentration of 7 mg/mL. Finally, samples were filtered through a 0.45 μm Millipore membrane, and 10 μL were injected in the chromatograph.

The presence of rutin and hyperoside, previously identified in this plant (Chiang et al., 2004; Kviecinski et al., 2011; Wu et al., 2007), was confirmed by comparing their retention times, UV spectra and spiking samples with a known concentration

Table 1 Storage conditions employed in the stability testing.

Storage Condition	Relative Humidity (%)	Temperature (°C)	Sampling time (month)						
			0.5	1	2	3	4	5	6
Room temperature[a]	63.5	25			x		x		x
Accelerated stability[a]	75	40	x	x	x	x	x	x	x
Refrigerator[b]	80	14			x		x		x

[a] Analysis of open and sealed containers.
[b] Analysis of sealed container (close).

of analytic standards. The concentration of polyacetylene isolated from the ethyl acetate fraction of the crude extract was also monitored. Analytic curves were constructed in order to calculate the concentration of the three compounds identified in the chromatogram of the extract.

2.6. Color analysis

Color is a relevant quality attribute of dried bioproducts. Color changes during stability studies can be related with changes in product composition due to chemical or biochemical reactions. The color changes of the dried products as a function of storage time under various temperatures and R.H. were analyzed in the Color Quest XE colorimeter (Hunter Lab, Riston, Virginia, EUA), using the reflectance-specular excluded mode (RSEX), D65 as illuminant and a 10° observer angle. Samples were placed in quartz cells and measurements of lightness L^* and chromaticity ($+a^*$ = red e $-a^*$ = green) e b^* ($+b^*$ = yellow e $-b^*$ = blue) were carried out. Those parameters were employed to calculate the cylindrical parameters (Croma, C^*) and Hue angle according to Eqs. (1) and (2). Samples were weighted in an analytic balance (Metter Toledo, Model AG204, Switzerland) and dissolved in 30 mL of distilled water, stirred for 2 h at 500 rpm (mag-multi, Marte, Brazil) and centrifugated at 7000 rpm for 15 min in a Eppendorf centrifuge 5430R, the supernatant was separated and measured.

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$H^\circ = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

3. Results and discussion

3.1. Concentration of the marker compounds during stability testing

Fig. 1 shows the concentrations of rutin, hyperoside and an unidentified polyacetylene in samples stored at different conditions during the whole stability testing period. Significant changes in the concentration of the markers compounds were observed among samples placed in open- and sealed containers. A sudden decrease in the concentration of all marker substances can be seen for samples kept in open containers compared to the ones stored in sealed sachets. With regard to the type of marker compound, it was observed that the polyacetylene suffered higher degradation especially when stored at the higher temperature (40 °C); while samples conserved into sealed sachets at 14 °C and at room temperature (25 °C) maintain product stability for one year, with no significant changes in the concentration of any marker substances. These results demonstrate that the storage conditions and packing design have significant impact on the stability of phytopharmaceutical compositions. Under correct storage conditions it is possible to guarantee the stability of the spray dried *B. pilosa* extract for at least one year.

3.2. Moisture uptake

The moisture uptake of samples under the different storage conditions is presented in Fig. 2. Samples kept in open containers showed an initial burst of moisture uptake followed by stabilization which could be related to saturation. On the other hand, samples stored into sealed containers did not show significant changes in product moisture during the stability testing period.

Degradation of the marker compounds were shown to be linked to moisture uptake, since samples stored in open containers, which presented more moisture uptake, showed a drastic decrease in the concentration. These results emphasize the importance of a correct selection of the packaging and storage conditions to preserve the concentration of the bioactive compounds in dried phytopharmaceutical compositions.

The drying carriers employed in the spray drying feed compositions could also influence the product moisture uptake, since colloidal silicon dioxide and microcrystalline cellulose are hygroscopic materials (Rowe et al., 2006). Colloidal silicon dioxide can absorb large quantities of water without liquefying. This material is commonly employed as a drying carrier due to its large specific surface area and high glass transition temperature which improve drying performance mainly for highly sticky products such as herbal extracts (Bott et al., 2010). Microcrystalline cellulose is useful as binder/diluent in tableting and to improve the flow properties of the phytopharmaceutical powders, which could be used as raw material for wet granulation and direct compression (Rowe et al., 2006).

3.3. Color variation

Color is an important attribute for the acceptance of food and nutraceutical products by consumers. Plant extracts are complex mixtures of different compounds and many of them are prone to oxidation and hydrolysis reactions, which can reflect in color changes. Since many degradation reactions are linked to water presence, the moisture adsorption can alter the color appearance of samples during storage. Indeed, samples placed in open containers, which present higher moisture uptake, get

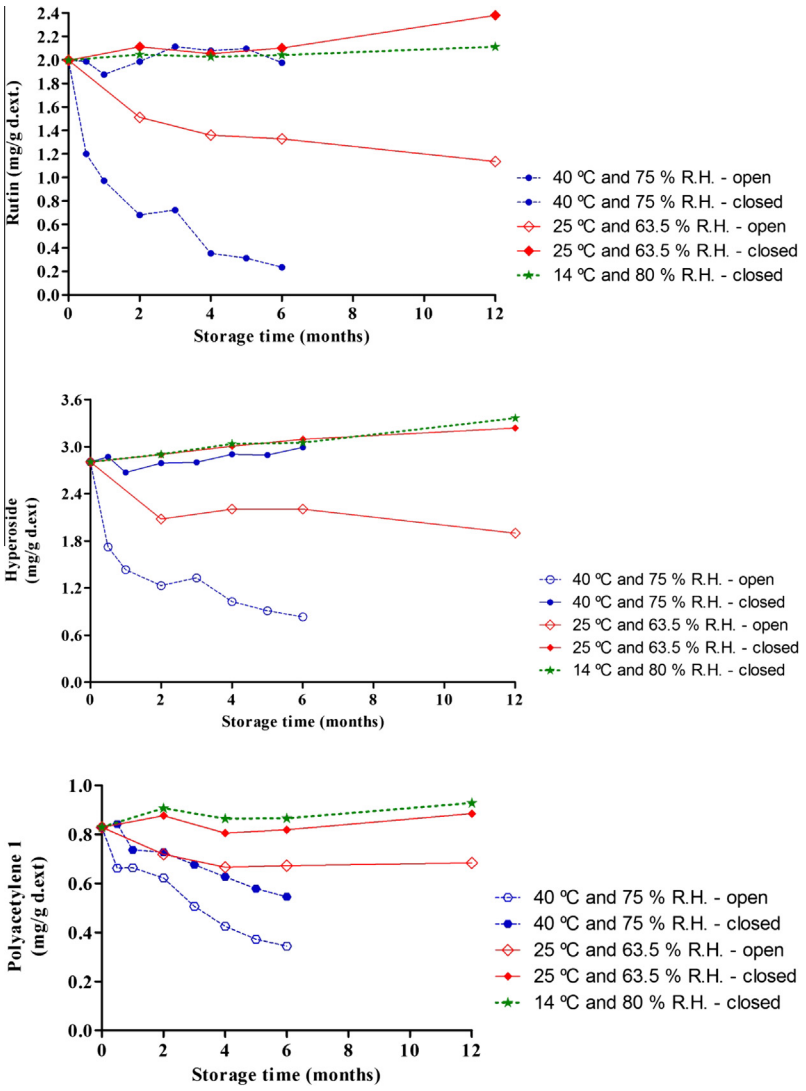

Figure 1 Concentration of rutin (a), hyperoside (b) and a polyacetylene (c) in spray dried extract stored at different temperatures and relative humidity.

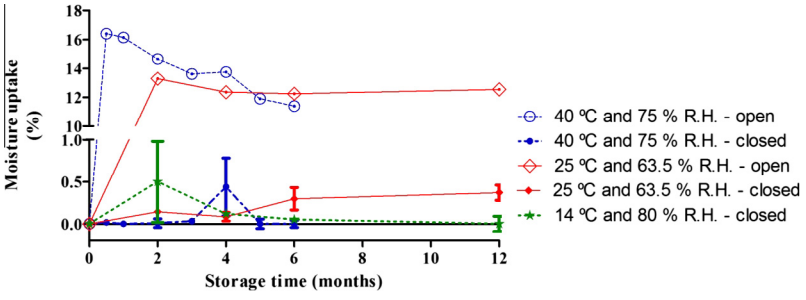

Figure 2 Weight variation of samples placed in open and close containers storage at different temperatures and Relative Humidity.

dark (browning) as a function of the storage time (see Fig. 3). The moisture content could affect the mobility of the molecular system which is directly related to the velocity of the degradation reactions. Moraga et al. (2012), determined the critical water content and critical water activity that should be maintained in freeze dried grapefruit powder, in order to conserve the glassy state of the amorphous matrix and therefore to increase product stability.

The crystalline state of solid materials also influences its stability and solubility (Souza et al., 2013). In the amorphous state, molecular mobility of the matrix and bioactive compounds is accelerated significantly, increasing the rate of occurrence of

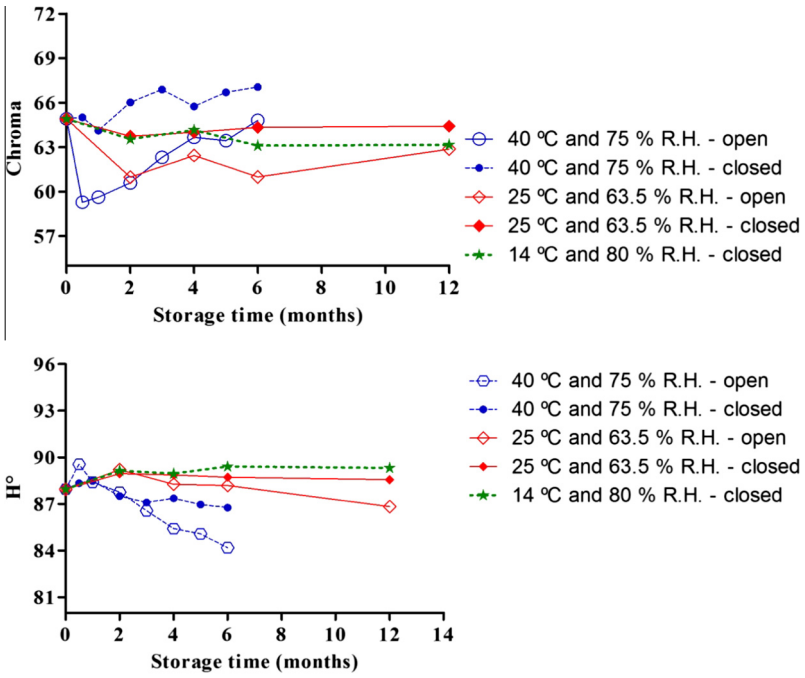

Figure 3 Color change of *Bidens pilosa* L. dried extracts at different storage conditions determined by the chroma and Hue angle.

caking, agglomeration and browning reactions among others (Bhandari and Howes, 1999; Fang and Bhandari, 2011). The effect of different drying carriers in the degree of crystallinity of *B. pilosa* dried extract was reported in a previous work by Cortés-Rojas and Oliveira (2012) where the product mixture containing Aerosil® and microcrystalline cellulose as drying carriers showed a moderate state of crystallinity. The proportion of microcrystalline cellulose added to the composition affects the degree of crystallinity of the final product (Cano-Chauca et al., 2005).

4. Conclusion

The concentration of the chemical markers did not change significantly in the refrigerator and in the moderate storage conditions when the sample was placed in sealed sachets. The unidentified polyacetylene was the compound with a higher degradation rate. With regard to color, darker hues were observed at higher temperatures and sampling times, which could be linked to the moisture gain. Results showed that the product could be stored up to one year in sealed sachets at 14 °C, 80% U.R. or at 25C 63.5% R.H. without significant changes in concentrations of monitored marker compounds. This study highlights the significant impact of packaging method and storage conditions in the maintenance of stability of dried phytopharmaceutical compositions from *B. pilosa*.

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