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Propolis encapsulation by spray drying: Characterization and stability

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ABSTRACT

Propolis extracts have shown to possess several health beneficial properties attributed to their phenol composition. Several pharmaceutical formulations are based on propolis as active ingredient and it could also be a potential component of functional foods. The main drawback for its application as food ingredient is its low water solubility, strong taste and aroma. In this work, an encapsulated Argentine alcohol-free propolis powder was obtained by spray drying, by using different maltodextrin matrices, with or without added gums. Besides, physicochemical characterization of powders and biocompound stability studies towards humidification at different water activities were done. Galangin and pinocembrin were the major components identified by HPLC in propolis before and after the drying process. The SEM images analysis showed that the addition of gums improved the particles integrity and size homogeneity. Furthermore, a higher degree of encapsulation of some polyphenols (such as quercetin), higher antioxidant activity measured by the reducing power assay, and higher physical stability toward humidification and physical collapse were also achieved especially in gum-added systems. The use of propolis as an encapsulated powdered additive widens its alcohol-free dosage applications.

1. Introduction

Propolis is a natural resinous mixture produced by honey bees from substances collected from parts of plants, buds, and exudates. Propolis extracts have shown to possess several health beneficial properties, such as antimicrobial, anti-inflammatory, healing, anesthetic, anticariogenic, antiviral, anticarcinogenic and antioxidant (Bodini, Sobral, Favaro-Trindade, & Carvalho, 2013; Chaillou & Nazareno, 2009). These properties are due to their chemical composition, more specifically to the presence of phenolic compounds, such as flavonoid aglycones, phenolic acids and their esters, aldehydes and ketones (Bodini et al., 2013). Isla, Nieva Moreno, Sampietro, and Vattuone (2001) found a positive correlation between flavonoid content of some Argentine propolis and percentage of inhibition of malon dialdehyde (MDA) by lipid oxidation. Although some specific propolis like Brazilian propolis type 6 or geopropolis lacks on phenolic acids and/or flavonoids compounds (Da Cunha et al., 2013; Duarte et al., 2003; Huang, Zhang, Wang, Li & Hu, 2014), in general the flavonoid content represents the major propolis components, and it was proposed as a good marker of propolis quality (Gardana, Scaglianti, Pietta, & Simonetti, 2007; Sampietro, Sampietro Vattuone, & Vattuone, 2016).

Several pharmaceutical formulations are based on propolis as active ingredient and it could also be a potential component of functional foods. Nevertheless, the application of propolis as a food ingredient is limited by its alcohol solubility, strong taste and aroma (Bodini et al., 2013; Nori et al., 2011).

Propolis encapsulation by spray-drying appears as an option to avoid undesirable sensory characteristics, protect bioactivity and widen the dosage by a water soluble encapsulation matrix (Bruschi, Cardoso, Lucchesi, & Gremião, 2003; Da Silva et al., 2013). Since bioactive compounds often present very low solubilities and bioavailabilities due to their hydrophobic character, spray-dried powder shows a considerable importance as it enhances the flavonoid compounds' solubility and absorption (Di Battista, Constenla, Ramírez-Rigo, & Piña, 2015; Fujimori et al., 2016), besides of reducing their thermal degradation (Pang, Mashitah,

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Yusoff, & Gimbun, 2014). Among all water-soluble matrices, maltodextrin is commonly used as a spray-drying agent for its high solubility and good bioactive compounds' retention (Franceschinis, Salvatori, Sosa, & Schebor, 2015; Pang et al., 2014). This property has been related to its rate of dehydration, which produces a rapid formation of dense skin and a good protection of core ingredient against oxygen transfer and possible deterioration (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007; Matsuno & Adachi, 1993). The physicochemical properties of powders and the retention of bioactive compounds can be modified by the addition of another ingredient (Da Silva et al., 2013; Krishnan, Kshirsagar, & Singhal, 2005). The use of gums as second matrix ingredients has proved to change release-kinetics in several encapsulation systems (Guan & Zhong, 2015; Kim, Choi, Kim, & Lim, 2015). Krishnan et al. (2005) showed that a blend of Arabic gum and maltodextrin protected cardamom oleoresin better than Arabic gum alone. Similarly, spray-dried powders with modified wall materials (tapioca starch and maltodextrin) showed differences in water solubility, surface morphology and β-carotene retention efficiency (Loksuwan, 2007).

In this context, the aim of this work was to obtain an encapsulated Argentine alcohol-free propolis (from a Biosphere Reserve) powder by spray drying, by using different maltodextrin matrices. A non-conventional galactomannan gum (*vinal* gum) and Arabic gum were employed as secondary encapsulation agents. The physicochemical characteristics and the antioxidant capacity of powders were determined and the main polyphenol compounds were quantified prior and after encapsulation.

2. Materials and methods

2.1. Materials

Propolis from Tigre (*Delta del Paraná* Biosphere Reserve, declared protected zone by UNESCO in 2000, unesco.org), was studied. It was obtained by honey bees from the buds and resin of many trees: poplar (*Populus alba*), willow (*Salix* spp.), cockspur coral tree (*Erythrina crista-galli*), Australian acacia (*Acacia mearnsii*) and pecan (*Carya illinoinensis*) (Burkart, 1957; Kalesnik & Aceñolaza, 2008). Maltodextrin (MD) dextrose equivalent 13 from Saporiti S.A. (Buenos Aires, Argentina), and Arabic gum from Biopack S.A. (Zárate, Argentina) were also employed.

2.1.1. Propolis purification

14 g of propolis were dissolved in 100 mL of ethanol and stirred for 24 h. Then it was filtered with a Buchner doing vacuum with 0.42 μm pore size paper filter. In order to remove all remaining wax, the ethanolic extract was kept at freezer (−20 °C) for 10 h and centrifuged at −5 °C at 4500 rpm for 10 min twice. The clear supernatant was evaporated in rotavap at 40 °C to a final volume of 40 mL. The final concentration of the ethanolic extract was 0.123 g/mL of purified propolis.

2.1.2. Vinal gum extraction and purification

Separation of *vinal (Prosopis ruscifolia)* seeds, extraction and purification of the gum were done according to previous work (Busch, Kolender, Santagapita, & Buera, 2015). Briefly, seeds were separated by milling the pods and passing through several sieves. Then, the endosperm was manually separated after alkaline treatment (Chaires-Martinez, Salazar-Montoya, & Ramos-Ramírez, 2008). The purification was done through water solubilization of the endosperm and flocculation of the gum into absolute ethanol twice. *Vinal* gum was freeze-dried by using a Heto Holten A/S, cooling trap model CT 110 freeze-dryer (Heto Lab Equipment, Alleroed, Denmark) operating at a condenser plate temperature of −111 °C, a chamber pressure of 30 Pa, and shelf temperature of 25 °C. *Vinal* gum has molecular weight of 1.43 ± 0.04 × 10^6 Da (viscometric), a mannose/galactose ratio determined by GC-MS of 1.6 and an apparent viscosity of 600 mPa s at 0.1 g/100 mL (Busch et al., 2015).

2.2. Encapsulation of propolis by spray drying

Three systems were prepared: maltodextrin propolis system without added gum (w-oG), maltodextrin - *vinal* gum propolis system (VG), and maltodextrin - Arabic gum propolis system (GA). Each system was prepared by stirring 30 g of MD, 0.3 g of Arabic gum or *vinal* in 100 mL of bi-distilled water at 500 rpm for 24 h. Then, each system was homogenized by an Ultra-Turrax T18 (IKA, Königswinter, Germany) at 15,000 rpm for 2 min and then 10 mL of the propolis ethanolic extract (0.123 g/mL solids) was added dropwisely, and suspended in the Ultra-Turrax for 2 min more. Each system was filtered twice to get rid of remaining solids, in order to prevent the clogging of the spray dryer nozzle. The operational conditions of a mini spray dryer (Büchi B290, Flawil, Switzerland) were: flow rate: 8 mL/min; air pressure: 3.2 kPa; nozzle diameter: 1.5 mm; inlet temperature: 120 °C. Outlet temperatures were between 70 °C and 74 °C for the three systems.

2.3. Propolis compounds analyzed by HPLC

A high-performance liquid chromatograph Agilent series 1100 equipped with UV detector was used (Agilent Technologies, Waldbronn, Germany). Separation was done by a purosphere STAR RP- 18 endcapped; 5 μm 150 × 4.6 mm column (Merck Millipore, Darmstadt, Germany). Temperature of column oven was set at 25 °C, flow rate was 0.7 mL/min, injection volume for both standards and extracts was 10 μL, and detection was performed at 290 nm. Elution was performed using acetonitrile-1% phosphoric acid solvent system using a linear gradient. Calibration curves for each particular standard (HPLC quality) were done using caffeic acid, p-coumaric acid, ferulic acid, quercetin, cinnamic acid, apigenin, naringenin and galangin (from Sigma-Aldrich Co., St. Louis, MO, USA); chrysin and pinocembrin (Fluka, from Sigma-Aldrich Co.); and pinocembrin derivate (pinocembrin-7-methylether, Roth, from Carl Roth GmbH, Karlsruhe, Germany). The encapsulation efficiency (called % encapsulation) was calculated by equation (1).

$$\%encapsulation_i = \frac{X_i \cdot 100}{Y_i} \quad (1)$$

where X_i is the amount of each compound found in the spray dried powder (mg/g of dry powder), and Y_i is the quantity of that compound in the propolis extract added to the solution entering the spray dryer (mg/g of solids).

2.4. Physicochemical characterization of powders

2.4.1. Water dispersibility (WD)

WD was evaluated by suspending 0.5 g of the spray-dried systems in 50 mL of bi-distilled water, stirring 5 min (vortex), and centrifuging at 5000 rpm for 5 min. A 20 mL supernatant aliquot was dried in oven at 105 °C for 2 h (adapted from Da Silva et al., 2013). WD was calculated following equation (2).

$$WD\ (\%) = \frac{weight\ (g) of\ solid\ supernatant\ \times 2.5}{weight\ of\ sample\ (g)} \quad (2)$$

2.4.2. Bulk density

Bulk density was determined by the tapping method. One gram of powder was loosely weighed into 10 mL graduate cylinder. The cylinder containing the powder was tapped on a vortex to a constant volume (Sablani, Shrestha, & Bhandari, 2008).

2.4.3. Hygroscopicity

Aliquots of the powders were placed into Petri dishes at 25 °C in a hermetic container with a saturated solution of NaCl (75% RH). After 7 d, hygroscopicity was evaluated (by weighing samples) and expressed as follows: g of water absorbed/100 g of dry system (Saikia, Mahnot, & Mahanta, 2015).

2.4.4. Water activity (a_w)

An electronic dew point water activity meter Aqualab Series 3 (Decagon Devices, Pullman, WA, USA) was used to determine water activity. Each spray-dried system was determined twice.

2.4.5. Water content

Karl Fisher titration was carried out with a Karl Fischer titrator DL 31 from Mettler-Toledo (Zurich, Switzerland), applying the one-component technique with Hydranal Titrant Composite 5 from Riedel-de Haën (Seelze, Germany). Pure methanol was used as solvent, and they were purchased from Merck. Sample sizes were approximately 100 mg, and standard deviation was calculated from three replicate measurements.

2.5. SEM images

Surfaces of samples were observed with a field-emission scanning electron microscope Supra 35 VP Carl Zeiss (Oberkochen, Germany) at an accelerating voltage of 1 kV. Before imaging samples were mounted on sample holder using double sided conductive carbon tape.

2.6. Images analysis

Segmentation of particles was performed via a combination of different image-processing tools. These include: median filter, Canny edge detection, Otsu thresholding, morphological operations such as erosion, dilation, opening and closing, flood-fill operations and watershed segmentation. This process is interactive: the user has to tag the correctly segmented particles. The area of each particle was estimated by counting the pixels inside the segmented particle, and its perimeter by counting the pixels on the border; both quantities were rescaled to squared microns and microns, respectively. The circularity is equal to 1 when the particle is a circle. The circularity of each particle was calculated by means of the following formula (3):

$$Circularity = \frac{4 \times \pi \times area}{perimeter^2} \quad (3)$$

Two images were studied for each system, by dividing the image in 8 non-overlapping sections (rectangles), plus 9 sections (rectangles) overlapping on the former ones, so as not to miss recognition of particles on the borders of the sections. Each section was analyzed by 7 routines (recognition methods) composed of different combinations of the image-processing tools described above, which take into consideration different aspects of the particles. Upon comparison of (for example) 2 routines, one same particle may be recognized by both routines, it may be recognized by one of them, or it may be missed by both. There are also false recognitions. In the 7 runs executed on a section, when a routine recognizes a new particle, this is presented to the user to tag as correct or incorrect. The final result is the addition of the particles recognized by the 7 routines. At least 180 particles were analyzed for each system.

2.7. Antioxidant activity assays

2.7.1. Free radical scavenging by DPPH

The reaction mixture was prepared by adding 50 μL of dissolved powder to 1.95 mL of an ethanolic solution of DPPH radical with an absorbance equal to 1 at 517 nm (Chaillou & Nazareno, 2009). The decrease in absorbance at 517 nm was determined spectrophotometrically after 30 min of reaction. The percentage inhibition of the radical by the samples, radical scavenging activity (RSA), was calculated and expressed as mg of gallic acid (AG)/g of powder. The surface % RSA was measured by the same DPPH assay, but using 50 μL of an ethanolic solution (3 mg of powder in 2 mL of ethanol, stirred for 15 min and centrifugated at 500 rpm for 5 min).

2.7.2. Reducing power by FRAP

The ferric reducing ability of samples was determined as described by Benzie and Strain (1996). The reducing power was expressed as mmol/L of $FeSO_4$/g of powder.

2.7.3. Total polyphenols by Folin Ciocalteu

Total polyphenols contents were determined by using the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) and expressed as mg AG/g powder.

2.8. Humidification treatment

Samples (1.0 g) were exposed to relative vapor pressure of saturated solutions of water activity (a_w) of 0.11 (LiCl), 0.22 (K_2CO_3), 0.33 ($MgCl_2$), 0.52 ($MgNO_3$), 0.75 (NaCl) and 0.84 (KCl) (Greenspan, 1977) until they reach the equilibrium in vacuum desiccators at 25 (±1 °C) for approximately 10 d.

All the samples were used for water sorption isotherm and differential scanning calorimetry analysis, as described afterwards. Samples equilibrated at 0.52, 0.75 and 0.84 were analyzed for free radical scavenging activity by DPPH.

2.9. Water sorption isotherm

The water content and water activity (a_w) of equilibrated samples in the a_w range 0.11–0.84 were determined for obtain the water sorption isotherm. GAB (Guggenheim–Anderson–de Boer, Da Silva et al., 2013) equation (4) was used to correlate the equilibrium water content of the samples with each corresponding water activity.

$$Y = \frac{q_m \cdot C_1 \cdot k \cdot X}{((1 - (k \cdot X)) \cdot (1 - k \cdot X) + (C_1 \cdot K \cdot X))} \quad (4)$$

where Y is the water content (dry basis); X is the water activity; q_m is the moisture content needed to cover the entire surface with an unimolecular layer. The Guggenheim constant, C_1, is related to the heat of sorption of the first layer on primary sites. The parameter k is a factor correcting properties of the multilayer molecules with respect to the bulk liquid.

2.10. Differential scanning calorimetry

A differential scanning calorimeter (DSC) Mettler-Toledo equipment (model 822, Mettler Toledo AG, Greifensee, Switzerland) with a STARe Thermal Analysis System version 8 software (Mettler Toledo AG) were used for all measurements. The

instrument was calibrated with indium and zinc. Samples (5–15 mg) humidified at different a_w were placed in 40 μL hermetically sealed aluminum pans (Mettler-Toledo AG). An empty pan was used as a reference. All the experiments were conducted under nitrogen atmosphere. Each sample was heated at a rate of 10 K/min. Glass transition temperatures (T_g) were recorded as the onset of discontinuity in the curves of heat-flow versus temperature. T_g values as a function of water mass fraction was modeled by the Gordon and Taylor (1952) equation, equation (5).

$$Y = \frac{W_1 \cdot T_{g1} + kW_2 \cdot T_{g2}}{W_1 + kW_2} \quad (5)$$

Where W_1–W_2 are mass fractions, and T_{g1}–T_{g2} are glass transition temperatures (onset value) of the pure components. The adjustable parameter *k* expresses the degree of curvature of the T_g-composition dependence.

2.11. Photographs by computer vision system (CVS)

The CVS consisted of three elements: a lighting system, a digital camera and a personal computer. The obtention of the images parameters were described by Agudelo-Laverde, Acevedo, Schebor, and Buera (2011). Briefly, the lighting system included a D65 lamp (this illuminant corresponds to solar irradiation) inside a grey chamber. The angle between the camera axis and the sample plane was 45° and the angle between the light source and the sample plane was 90°. A high-resolution (10.1 mega-pixel) digital camera, an EOS 40D (Canon Inc., Japan) was used, with an EF-S 60 mm f 2.8 macro lens (Canon Inc., Japan). The digital camera was operated in manual mode, with the lens aperture at f = 6.3 and speed 1/8 s (no zoom, no flash) to achieve high uniformity and repeatability. The collapse of material was determined macroscopically as the shrinkage due to the loss of the stabilizing inner structure, volume contraction and change of color (Saavedra-Leos, Leyva-Porras, Araujo-Díaz, Toxqui-Terán, & Borrás-Enríquez, 2015). During collapse, open pores disappear and a highly viscous amorphous melt is obtained (Hartmann & Palzer, 2011).

2.12. Statistical analysis

The experimental data were fitted by the corresponding equations, by minimizing the square differences. One-way analysis of variance (ANOVA) with Tukey post-test using Prism v5 (GraphPad Software, Inc., San Diego, CA, USA) was used to analyze the differences between mean values for each model. When the analysis of variance indicated differences among means, a student test was performed to differentiate means with 95% confidence ($P < 0.05$). All analysis were conducted in triplicate.

3. Results and discussion

3.1. Polyphenolic compounds analyzed by HPLC

Propolis compounds in the purified propolis extract and in the resuspended spray-dried powders were identified and quantified by HPLC. Fig. 1 shows the HPLC chromatogram of the ethanolic extract of Argentine propolis from Tigre (Biosphere reserve - protected area). Furthermore, several polyphenolic compounds were identified: hydroxinamic acids with lower retention times (caffeic acid, coumaric acid, ferulic acid), flavonoid compounds with middle and higher retention time as polarity decreased (quercetin, apigenin, naringenin, chrysin, pinocembrin, galangin and pinocembrin derivate). Table 1 shows the relative amounts of the corresponding identified compounds. Our results were qualitatively in agreement with findings for other propolis from other Argentine regions. However, while pinocembrin was the major component in propolis from Santiago del Estero Province (Chaillou & Nazareno, 2009) and in Argentine propolis from non-specified areas (Gardana et al., 2007), in the propolis from Tigre galangin was the major component (289 ± 15 mg/g of purified propolis) followed by pinocembrin (180 ± 9 mg/g of purified propolis). The total polyphenol content of the original propolis extract was 866 ± 22 mg of AG/g of propolis. This value was in good agreement with Ethiopian propolis (365–1022 mg of AG/g, Dubero Sime, Atlabachew, Redi-Abshiro, & Zewde, 2015), and higher than those informed for other Argentine propolis (Santiago del Estero area, 42–253 mg of AG/g, Chaillou & Nazareno, 2009).

Fig. 2 shows the encapsulation degree for the major propolis compounds for each spray dried system, obtained by HPLC. The HPLC chromatograms are shown in Fig. S1. Although the global encapsulation degree, expressed as coumaric acid, was similar for the three systems, there were differences for each individual polyphenol compound. Quercetin showed a high encapsulation in both systems with added gum (VG and GA). This could be related to the flavonol structure of quercetin that could have stronger interactions with polymer gums structure (galactomannan from *vinal* gum (Busch et al., 2015) and arabinogalactan from Arabic gum (Zuidam & Nedović, 2010), respectively. On the other hand, some compounds (crysin, pinocembrin, galangin and pinocembrin) showed higher degree of encapsulation in GA system, which could be attributed to the well-known emulsifier ability of Arabic gum

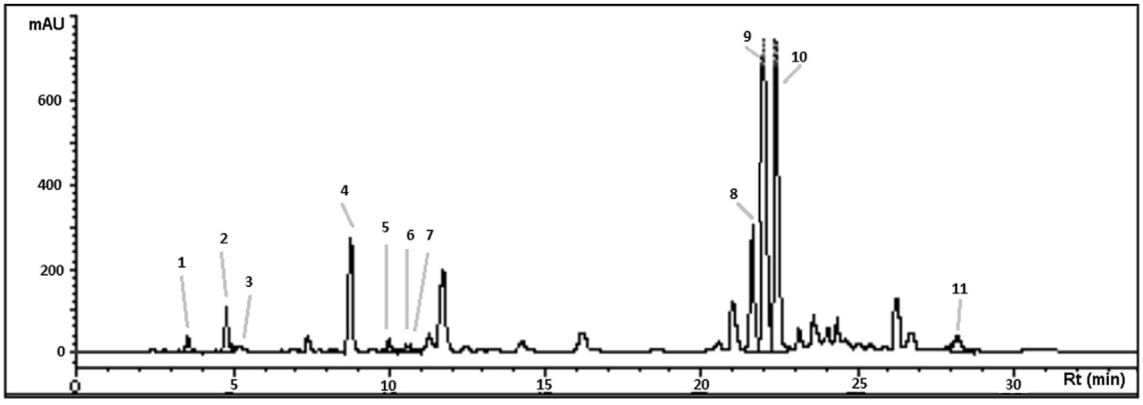

Fig. 1. HPLC chromatogram of Argentine propolis. Milliunits of absorbance (mAU) at 290 nm as a function of retention time (Rt, in min) of most significant phenolic compounds. See Table 1 for assignation.

Table 1
HPLC peak assignation, retention time and area quantification of Argentine propolis major compounds.

Peak number	Propolis compound	Retention time	mg/g of propolis[a]
1	Caffeic acid	3.56	7.4 ± 0.2
2	Coumaric acid	4.8	18.2 ± 0.3
3	Ferulic acid	5.03	ND
4	Quercetin	8.24	9.1 ± 0.2
5	Cinnamic acid	10.02	6.6 ± 0.1
6	Apigenin	10.55	ND
7	Naringenin	10.7	ND
8	Chrysin	21.6	96 ± 2
9	Pinocembrin	21.99	180 ± 9
10	Galangin	22.4	289 ± 15
11	Pinocembrin derivate	28.2	18.1 ± 0.6

[a] Mean ± standard deviation of triplicate determinations.

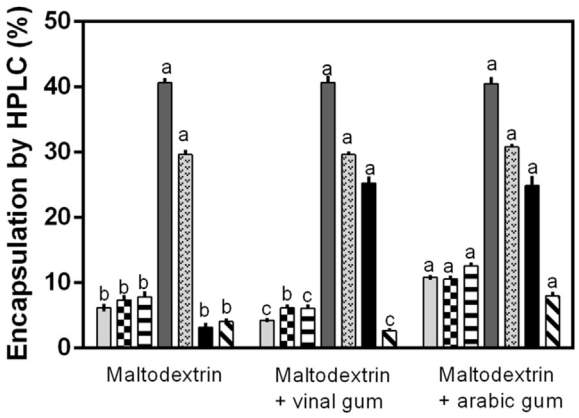

Fig. 2. Retention of propolis components by spray-drying encapsulation in three systems: w-oG (maltodextrin propolis system, without added gum); VG (maltodextrin - *vinal* gum propolis system) and GA (maltodextrin - Arabic gum propolis system). Data obtained by HPLC was expressed as the ratio between the obtained concentration in the dried powders and the amount present in the initial system (prior to spray drying). Different columns show different identified compounds: crysine (▭), pinocembrine (▭), galangine (▭), coumaric acid (▭), cinnamic acid (▭), quercetin (▭) and pinocembrine derivate (▭). Standard deviation values are included. For a given compound, significant difference between systems is indicated with different letters (a–c; $P < 0.05$).

(Gharsallaoui et al., 2007; Krishnan et al., 2005; Sarkar, Gupta, Variyar, Sharma, & Singhal, 2013).

3.2. Physicochemical characterization of the spray-dried powders

Table 2 shows the physicochemical properties of the obtained spray-dried powders and spray-drying yields (calculated as the ratio between the mass of obtained dried solids and initial total solids content entering in the spray dryer). As a general trend, the results of the studied characterization properties and yield were typical of spray-dried powders (Di Battista et al., 2015; Sablani et al., 2008; Saikia et al., 2015). In the present work, the systems with added gum (GA and VG) showed similar dispersibility in water and bulk density than the control system without gum (w-oG). The *vinal* gum containing system (VG) had the highest hygroscopicity, probably related to the lowest water content at the end of drying. Furthermore, the VG system had the lowest yield; this fact can be attributed to technical problems caused by the high viscosity of *vinal* gum solution (Busch et al., 2015) during spray drying and previous filtration.

Fig. 3 shows the obtained SEM images for the w-oG system and the schematic particles segmentation performed by the SEM-image analysis by some of the processing tools applied. SEM images of the other systems are shown in Fig. S2. The spray dried powders showed deformed spherical shape with extensive dented surface, which is attributable to the shrinkage of the particles during the drying process (Loksuwan, 2007). This behaviour has been already reported in other systems with maltodextrin and Arabic gum matrices (Di Battista et al., 2015; Krishnan et al., 2005). At first glance, all the three systems showed similar shape and morphology, and the particles surface presented no fissures. The continuous wall and fissure absence guarantee a good encapsulation-matrix barrier and a better core material protection (Da Silva et al., 2013; Di Battista et al., 2015).

Fig. 4a shows the size results from the SEM images analysis. The distribution of spray-dried particles according to their area is similar for the three studied systems. Nevertheless, if the size distribution is analyzed in the 0–2 μm^2 range, some differences could be observed. There is a higher frequency of smaller particles (smaller area) for the w-oG system, followed by the VG system. GA systems showed the biggest size and a peak at 16 μm^2. These results show that the addition of gum to the system affects the size of the obtained spray-dried powder. Fig. 4b shows the circularity of the analyzed SEM images. For a perfect circle the circularity would be equal to 1. It has to be noted that when the particles retain their integrity during drying, they become irregular during cooling due to the shrinkage promoted by the air contraction and consequent vacuum in their interior. However, when the particles are broken in the process, the fissures allow re-equilibration of air inside with the exterior and they have a higher circularity. Thus, higher circularity (in the range of 0.8–1) indicates a greater degree of broken particles. The circularity of the w-oG system showed a maximum in the 0.8–1 range, attributable to a higher frequency of broken particles.

3.3. Water sorption, glass transition temperature and color upon humidification

Fig. 5 shows the water sorption isotherm for the three systems and the glass transition temperature values (T_g) as a function of water mass fraction. System w-oG presented a water isotherm slightly shifted towards lower water content values at high a_w values. Water sorption isotherms were fitted using GAB equation, which was previously successfully used in maltodextrin systems obtained by spray drying (Da Silva et al., 2013). The GAB analysis provided the hydration limit of 0.070 ± 0.005, 0.097 ± 0.003 and 0.082 ± 0.004 g water/g sample. The C1 constants, related to the

Table 2
Physicochemical properties and yields of the propolis spray dried powders: w-oG (maltodextrin propolis system, without added gum); VG (maltodextrin-*vinal* gum propolis system) and GA (maltodextrin-Arabic gum propolis system).

	Water dispersibility (% w/w)	Bulk density (g/mL)	Hygroscopicity (% w/w)	a_w	Water content (% d.b.)	Spray drying yield (% w/w)[a]
w-oG	97 ± 3a	0.37 ± 0.02a	8.4 ± 0.1b	0.216 ± 0.003b	1.91 ± 0.06b	68
VG	100 ± 1a	0.33 ± 0.03a	9.1 ± 0.1a	0.216 ± 0.006b	1.64 ± 0.07c	60
GA	100 ± 3a	0.32 ± 0.02a	8.5 ± 0.4b	0.228 ± 0.003a	2.21 ± 0.04a	68

Mean ± standard deviation of triplicate determinations are informed. Different letters (a–c) indicate significant differences ($P < 0.05$) between means.
[a] Calculated as the ratio between the mass of obtained solids and initial total solids content entering in the spray dryer.

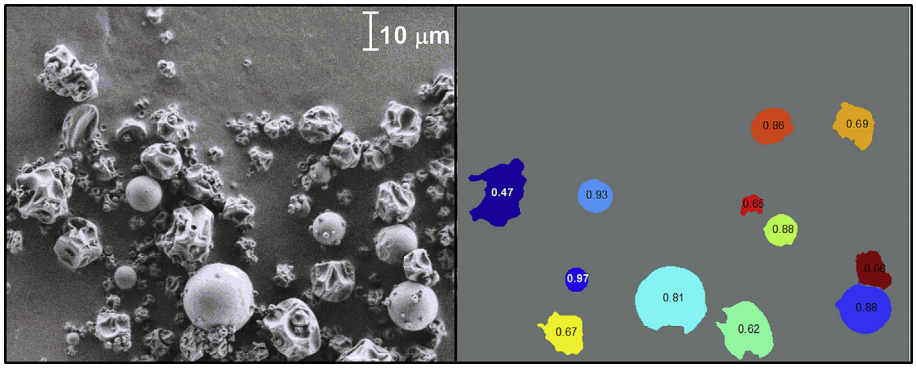

Fig. 3. SEM image and particle analysis. Left: SEM image of spray-dried propolis system (maltodextrin and propolis, without added gum). Right: selected particles segmented by image analysis using the processing tools. The number inside each particle indicates circularity, and the white patches show the recognized surface for each particle.

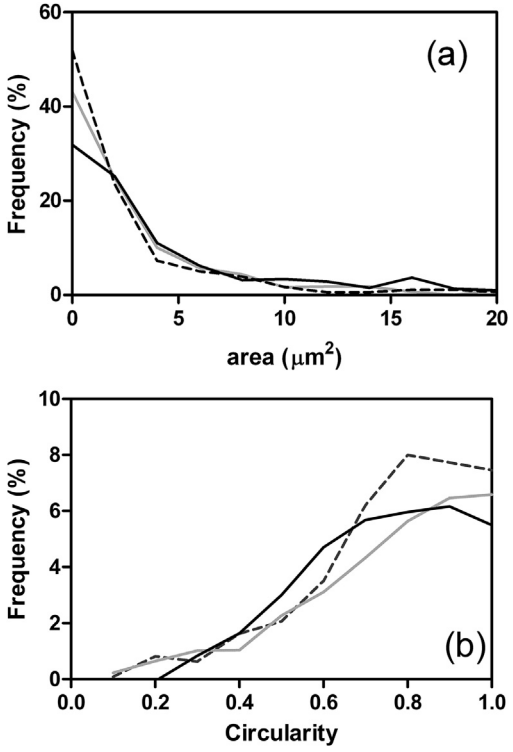

Fig. 4. Area (a) and circularity (b) of the spray-dried powders from envelope curves obtained by histograms analysis of SEM-images of three propolis spray dried systems: ---- w-oG (maltodextrin propolis system, without added gum), —— VG (maltodextrin-*vinal* gum propolis system), —— GA (maltodextrin - Arabic gum propolis system).

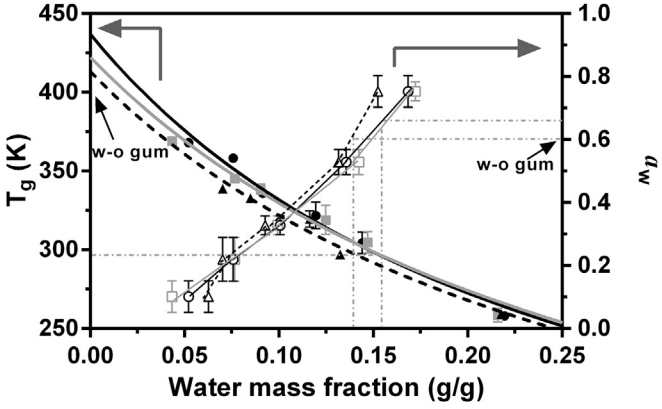

Fig. 5. Glass transition temperature values (filled symbols) fitted by Gordon & Taylor equation (left Y axis) and water sorption isotherm (unfilled symbols-right Y axis) for propolis spray dried powders. -△-, -▲- w-oG system (maltodextrin propolis system, without added gum); -□-, -■- VG system (maltodextrin-*vinal* gum propolis system); -●-, -○- GA system (maltodextrin-Arabic gum propolis system).

heat of sorption, were 33, 8.5 and 14.8 for the systems w-oG, VG and GA, respectively. The k values were similar for the three studied systems, in a range between 0.8 and 0.85 (no significant difference among them). Although the w-oG system showed higher equilibrium a_w value at a given water content (related to a left-shifted water sorption isotherm), the corresponding T_g value was lower than that of the added gum systems (GA and VG). A critical water activity value could be determined for each system as the upper limit of the stability a_w range established by the storage temperature (room temperature, 298 K, indicated by the horizontal dotted lines shown in Fig. 5). The critical a_w values were 0.6 for the systems wo-G and 0.67 for the systems with gums, corresponding to the water mass fractions 0.139 and 0.155 g, respectively. When stored at lower a_w than these values, each system would be physically stable.

It can be observed that the systems containing gums are expected to maintain their physical structure at higher water contents or a_w values than the systems w-oG. It is to be noted that these water contents are about twice of the hydration limits calculated by the GAB model, discussed before.

Fig. 6 shows the photographs of the studied systems humidified at different a_w values. In agreement with the observed stability range discussed in the previous section, at $a_w = 0.52$ the systems retained its powder appearance. Although the critical a_w values were lower than 0.75, at this a_w the systems showed slight incipient agglomeration and were a little darker in color (Fig. S3). It has to be noted that the macroscopic changes expected above the T_g value depend on both time and T-T_g (Buera et al., 2011), and in the time-frame and conditions of the experiments performed, the collapse of the systems stored at $a_w = 0.75$ was not evident. On the other side, macroscopic physical changes could be easily observed when humidified at $a_w = 0.84$, at which all three systems showed physical collapse. However, the systems GA and VG kept a better structure and were not completely fluid (Fig. 6), in comparison with the system w-oG.

It is interesting to note that the particle properties studied in relation to Figs. 3 and 4 (particle size distribution, morphology and circularity) are determinant to define the particles hygroscopicity, solubilization kinetics in water and the caking events, including agglomeration, compaction and sticking (Aguilera, Valle, & Karel, 1995), discussed in relation to Figs. 5 and 6. Pang et al. (2014)

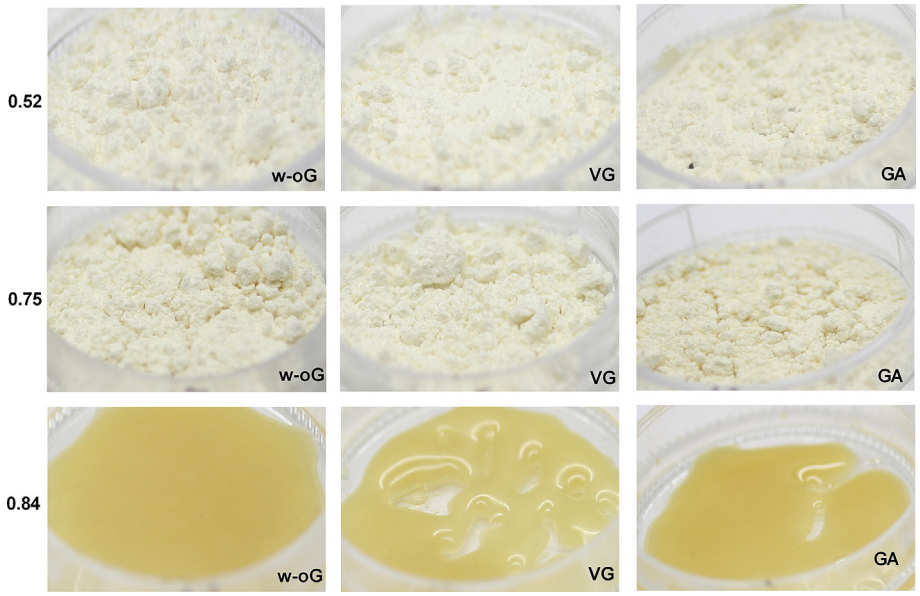

Fig. 6. Photograph of spray dried powders humidified at water activities of 0.52, 0.75 and 0.84 during 15 d. Three systems were analyzed: w-oG (maltodextrin propolis system, without added gum); VG (*vinal* gum and maltodextrin propolis system) and GA (Arabic gum and maltodextrin propolis system).

Table 3
Antioxidant activity of propolis powders immediately after spray-drying: total polyphenols content (TP) by Folin-Ciocalteu, radical scavenging activity by DPPH assay (RSA), both expressed as mg of gallic acid (AG) and reducing power (RP) by FRAP assay expressed as mM of $FeSO_4$. All three antioxidant assays are expressed by mass of dry powder.

	RP (mM $FeSO_4$/g)	TP (mg de AG/g)			RSA (mg AG/g)		
	Total	Total	Encapsulated %	Surface %	Total	Encapsulated %	Surface %
w-oG[a]	10.7 ± 0.6c	1.67 ± 0.02b	89 ± 9	11 ± 1	0.58 ± 0.11b	86 ± 9	14 ± 2
VG	12.5 ± 0.8b	1.70 ± 0.06b	89 ± 9	11 ± 1	0.55 ± 0.09b	81 ± 8	19 ± 3
GA	14.7 ± 0.7a	2.60 ± 0.05a	93 ± 7	7.4 ± 0.6	0.79 ± 0.06a	84 ± 9	16 ± 2

Mean ± standard deviation of triplicate determinations are informed. Different letters (a–c) on columns indicate significant differences ($P < 0.05$) for a given antioxidant assay.
[a] w-oG: maltodextrin propolis system, without added gum; VG: maltodextrin - *vinal* gum propolis system; GA: maltodextrin - Arabic gum propolis system.

related a less dented morphology to a better flowability in spray-dried powders from a higher solid content or with higher viscosity emulsion. The addition of gum to the system may increase particle skin flexibility during drying, avoiding fissures formation, and the surfaces appear to be flexible enough to allow the particles to collapse after the maximum expansion point was reached (Wang & Langrish, 2010).

3.4. Propolis bioactive compounds: reducing power, total polyphenols and radical scavenging activity

Table 3 shows the antioxidant activity for the three spray-dried systems studied through their reducing power (RP) by FRAP, their total polyphenol (TP) content by Folin-Ciocalteu, and their radical scavenging activity (RSA) by the DPPH assay. The addition of Arabic gum produced a higher retention of reducing power, polyphenol and radical scavenging propolis compounds. Da Silva et al. (2013) also found that Arabic gum was better to preserve the bioactive compounds during spray-drying than other carriers. Besides, there were less free polyphenols on the surface of the particles, which has been attributed to a greater physical protection ability of the carrier (Da Silva et al., 2013). The addition of *vinal* gum showed to increase the reducing power propolis compounds' retention, nonetheless VG showed similar results to w-oG for TP and RSA.

Fig. 7 shows the radical scavenging activity retention for the three systems after humidification at three different a_w. At $a_w = 0.52$ the retention was the highest, followed by the systems exposed at $a_w = 0.84$. At $a_w = 0.75$ the retention was the lowest for the three systems. The radical scavenging activity is better protected at lower a_w. However, a higher bioactive compounds retention at $a_w = 0.84$ than at $a_w = 0.75$, shows a compromise between the chemical stability provided by the maintenance in the glassy state (at $a_w = 0.52$). At $a_w = 0.75$ the samples were close, but above the T_g and the porous structure could have accelerated

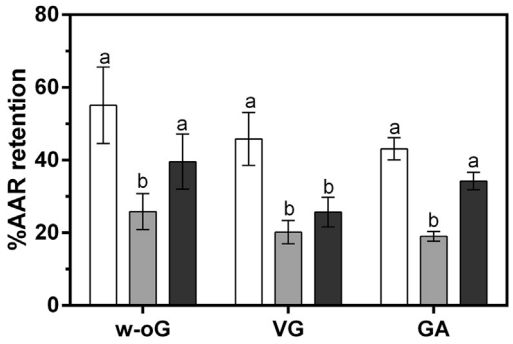

Fig. 7. Stability of powders in humidified systems at three water activities: 0.52 (white), 0.75 (grey) and 0.84 (black). The retention of the radical scavenging activity (RSA%) of the propolis was evaluated by DPPH assay and was calculated as the percent of initial radical scavenging activity (prior to humidification). Significant difference between systems is indicated with different letters (a–b; $P < 0.05$). w-oG: maltodextrin propolis system, without added gum; VG: maltodextrin-*vinal* gum propolis system; GA: maltodextrin - Arabic gum propolis system.

oxidative reactions. On the other side, for systems equilibrated at $a_w = 0.84$ the physical collapse promoted by the storage well above T_g has protected the polyphenols. It has been reported that structural collapse provides protection to biocompounds. Prado, Buera, and Elizalde (2006) found an inverse correlation between collapse and degradation rate constants for β-carotene. They found that the molecular mobility of the matrix is not rate limiting for the degradation of biocompounds, since many factors such as microstructure and porosity of the polymeric matrix may be more important as modifiers of kinetic reactions. These characteristic could be changing between $a_w = 0.75$ and $a_w = 0.84$ producing a biocompound retention difference.

4. Conclusions

Propolis polyphenols from a Biosphere Reserve were encapsulated by spray-drying, which allows to broaden propolis applications, since a free of alcohol dosage can be produced. Different encapsulation degrees were achieved, depending on the matrix composition and on the structure of the encapsulated compound. The addition of gums to the matrix formulation improved the encapsulation of some polyphenols (especially quercitin) during spray-drying.

Powders of higher T_g values were generated in the presence of gums, with particles of more homogeneous size, less proportion of broken particles, more skin flexibility (avoiding fissures formation) in comparison to systems without gum, as observed by image analysis. The studied particle properties (particle size distribution, morphology and circularity) were determinant to define the particle hygroscopicity and solubilization kinetics in water. Besides, sorption properties and T_g values allowed to define the powder stability and preservation conditions of the radical scavenging activity of propolis.

Structural collapse was evident in samples above their T_g values, which increased the retention of radical scavenging compounds, in comparison to those humidified in the proximities of T_g, at $a_w = 0.75$.

Thus, both macroscopic aspects and molecular events could be related for a better definition of the systems stability.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2016.08.055.

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