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Co-crystallized sucrose with propolis extract as a food ingredient: Powder characterization and antioxidant stability

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Abstract

Propolis possesses health beneficial properties due to its antioxidant compounds; however, its solubility in alcohol and its strong and unpleasant taste limit the use of propolis extract in foods. This study explores the encapsulation of a propolis ethanolic extract by co-crystallization in a sucrose matrix to eliminate the alcohol and to obtain a propolis powder with suitable technological properties and high antioxidant activity to be used as a food ingredient. The effect of the propolis extract on colour, moisture content, solubility, particle size distribution, flow properties and spectroscopic characteristics (FTIR) of the co-crystallized powders was determined. Polyphenols and flavonoids content and antioxidant capacity (ABTS and DPPH) during storage of the powders were also analysed. The propolis co-crystallized powders showed moisture contents below 2% and good flow properties, except at the higher content of the extract. The FTIR results showed that the presence of propolis did not alter the crystalline sucrose structure. Entrapment yields higher than 84% (flavonoids) and 78% (polyphenols) were obtained. During storage of the powders high stability of these compounds was observed at light, darkness and refrigeration conditions. Thus, co-crystallization technique constitutes a low cost alternative for the protection of bioactive compounds of propolis.

Keywords: Propolis, Antioxidants, Co-crystallization, Sucrose, Encapsulation

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1. Introduction

Propolis is a resinous, sticky, and coloured solid produced by bees (*Apis mellifera* L.) from beeswax and plant exudates. Bees use propolis as a defense by coating and strengthening the inside walls of the hive and by covering holes and cracks. Chemical composition of propolis depends on species of bees, geographical and botanical origin, harvest season and harvest conditions (Bueno-Silva, Marsola, Ikegaki, Alencar, & Rosalen, 2017; Maldonado et al., 2020; Papotti, Bertelli, & Bortolotti, 2012). Due to its chemical composition, propolis possesses bioactive properties, such as antioxidant, antimicrobial, antifungal, antiviral, anti-inflammatory, anticarcinogenic, antiallergenic, among others. The presence of phenolic compounds, such as flavonoid aglycones, phenolic acids and their esters, aldehydes and ketones, are the main responsible of these properties (Coelho et al., 2015; Cottica et al., 2015; Chan, Cheung, & Sze, 2013; Fangio, Orallo, Gende, & Churio, 2019; Salas et al., 2016; Yasar et al., 2016).

Propolis extract can be obtained from the raw material by grinding and subsequent steps of ethanol dissolution and filtration to remove wax and other organic impurities. Nowadays, application of propolis extract in foods is mainly as a natural antioxidant agent and preservative (Cottica et al., 2015; Santos, Estevinho, de Carvalho, da Silva Conceição, & de Castro Almeida, 2020; Vasilaki, Hatzikamari, Stagkos-Georgiadis, Goula, & Mourtzinos, 2019). The incorporation of propolis as a food ingredient would be an interesting alternative to increase the content of bioactive compounds. However, its fraction of volatile phenolic acid imparts a characteristic odor which, together with its strong and unpleasant taste, can negatively influence the sensory characteristics of food products. This reason and the low solubility in water limit the use of propolis as an ingredient in foods. Recently, sweet ingredients (honey and stevia) were used to mask its flavor in gummy jellys (Rivero et al., 2020) and microencapsulated propolis was applied in fish burgers (Spinelli, Conte, Lecce, Incoronato, & Del Nobile, 2015).

Encapsulation technology is a strategy to incorporate propolis extract as a food ingredient and to solve the adverse sensory and solubility aspects of this material.

Encapsulation is defined as the entrapment of a compound or a system inside a dispersed material for its immobilization, protection, controlled release, structuration and functionalization (Poncelet, 2006). Different techniques have been applied to encapsulate propolis bioactive compounds, among them, spray-drying (Andrade et al., 2018; Busch et al., 2017; Da Silva, Favaro-Trindade, de Alencar, Thomazini, & Balieiro, 2011), freeze-drying (Šturm et al., 2019), incorporation in a β-cyclodextrin cavity with subsequent freeze drying (Kalogeropoulos, Konteles, Troullidou, Mourtzinos, & Karathanos, 2009), emulsification-solvent evaporation (Durán, Marcato, Buffo, De Azevedo, & Esposito, 2007), complex coacervation (Nori et al., 2011), ionic gelation (Keskin, Keskin, & Kolayli, 2019), nanoprecipitation (do Nascimento et al., 2016) and a pH-induced one-step assembly method (Zhang et al., 2019). As far as we know, no research has been conducted on the encapsulation of ethanolic extract of propolis using co-crystallization technique. This process could be a simple and low-cost alternative to obtain a free-alcohol propolis powder without affecting its functional properties.

Through encapsulation by co-crystallization in sucrose matrices it is possible to obtain powders with good fluidity, solubility, wettability, compactibility and chemical stability (Bhandari & Hartel, 2002). Granulated sucrose structure is modified to be used as an encapsulation material, by changing from perfect crystals to irregular, agglomerated, and micro sized crystals where the active compound can be incorporated. Sucrose is an ideal material for transporting ingredients with functional activity considering its low hygroscopicity and high solubility and hydration capacity. In addition, sugar allows improving the sensory attributes of the products. Several researches were carried out in our laboratory to encapsulate bioactive compounds within the crystalline structure of sucrose for their protection: calcium lactate, magnesium sulfate and yerba mate extracts (Deladino, Anbinder, Navarro & Martino, 2007; López-Córdoba, Deladino, Agudelo-Mesa, & Martino, 2014, López-Córdoba et al., 2015), zinc sulfate (López-Córdoba, Gallo, Bucalá, Martino, & Navarro, 2016) and

glucose (López-Córdoba & Navarro, 2018). Other authors also applied co-crystallization to encapsulate cardamom oleoresin (Sardar & Singhal, 2013), marjoram extract (Sarabandi, Mahoonak, & Akbari, 2018), paprika oleoresin (Federzoni, Alvim, Fadini, Silva, & Queiroz, 2019), *Securigera securidaca* (L.) seed extract (Nik, Vazifedoost, Didar, & Hajirostamloo, 2019), *Basella rubra* extract (Karangutkar & Ananthanarayan, 2020). The present work involves finding the optimal formulations that maximize the load of propolis in the sucrose matrix, without losing its antioxidant properties during processing or storage. This would allow selecting the formulations that could be used as food ingredients with bioactive compounds by replacing the sugar, total or partially, in confectionery (candies) or in powder premixes of dairy (ice cream) or bakery products (cakes, cookies). Therefore, the objectives of this work were to encapsulate different contents of propolis extract by co-crystallization in a sucrose matrix and to evaluate the physicochemical properties of the powders and their antioxidant characteristics during storage.

2. Materials and Methods

2.1. Raw material and preparation of the propolis extract

Propolis was obtained from beehives located in Ignacio Correas (La Plata, 35º02'40''S, 57º50'08''W), center-east of Buenos Aires province (Argentina). The samples were kept frozen at -18°C and protected from light to prevent their natural oxidation. The propolis ethanolic extract (PEE) was obtained from the frozen crude propolis. First, 100 g of sample was grinded in a mortar until a powder was obtained. Then, the powder was dissolved in 900 mL of 96° (v/v) ethyl alcohol and stored for 3 d in an oven at 40°C, stirring 30 min every day. On the 4th d the solution was placed at 0°C for 2 h and filtered to remove waxes. Then, it was completed to a final volume of 1000 mL with 96º (v/v) ethyl alcohol, obtaining a 10 g/100 mL of PEE, which was preserved refrigerated in a dark container until use.

2.2. Preparation of the co-crystallized powders

The co-crystallized powders were prepared as described by Deladino et al. (2007). Commercial sucrose (50 g) (Ledesma, Argentina) was mixed with water (10 mL) and heated on a hot plate stirring with a vertical agitator (IKA Labortechnik, Germany). When a slight turbidity was detected in the syrup (indicating the beginning of the crystallization process), 10 mL ethanol (control sample) or the corresponding volume of PEE was added (Table 1). Immediately the mix was removed from the heat, maintaining the agitation for a few min to allow the ethanol evaporation. The solid obtained (co-crystallized) was transferred to a glass Petri dish and dried at 40°C in a forced air convection oven (Heratherm, Thermo Scientific, USA) for 24 h. Then, the dried co-crystallized agglomerates with propolis (CP) were reduced to a powder with a grinder and placed in dark glass containers protected from light with aluminum foil. Samples were stored in desiccators with silica gel until analysis.

2.3. Characterization of propolis co-crystallized powders

2.3.1. Colour

The colour of powders was measured with a chroma meter (CR-300, Minolta, Japan) calibrated with a standard white plate (Y = 93.2, x = 0.3133, y = 0.3192). Measurements were performed with illuminant D_{65} and 45°:0° measuring geometry. CIELab coordinates L*, a* and b* were determined: L* is lightness (0 = black, 100 = white), a* (- = greenness; + = redness) and b* (- = blueness; + = yellowness). Values are the average of at least three determinations. The total colour difference ΔE was calculated with respect to the coordinates (a_0*, b_0* y L_0*) that characterized the colour of the control sample:

$$\Delta E=\sqrt{(L_1-L_0)^2+(a_1-a_0)^2+(b_1-b_0)^2} \qquad (1)$$

2.3.2. Moisture

A weighed sample of powder was placed in a glass Petri dish and dried in a vacuum oven (DZF-6030A, Li Tekvo Instruments, China) at 70°C. After 24 h, the dish was removed from the oven, allowed cooling in a desiccator, and weighed. Then, the process was repeated until constant weight and the moisture percentage was calculated by triplicate (AOAC, 1998).

2.3.3. Solubility

The method of Cano-Chauca, Stringheta, Ramos, & Cal-Vidal (2005) with some modifications was used. The powder (1 g) was added to 100 mL of distilled water stirring with a magnetic stirrer at 400 rpm for 4 min at 20°C. The resulting solution was centrifuged at 3000 × *g* for 4 min. A 25 mL aliquot of the supernatant was transferred to a pre-weighed Petri dish and dried in an oven at 105°C for 5 h. The solubility in water was calculated from the weight of dried solid matter as a percentage of the initial powder. The solubilization time of the powders was also determined by adding 1 g of sample to 10 mL of distilled water under constant stirring. The end point was considered when no particles in suspension were observed. The test was repeated 3 times and the results averaged.

2.3.4. Particle size distribution

The analysis was carried out by triplicate using 4 sieves (Test Sieve, England), with mesh sizes from 2 to 0.5 mm. Particle sizes of the powders were obtained from serial sieving, with lateral, vertical and circular movements for 5 min, or until no changes were observed in any fraction of the sieve (< 0.5% of the total weight of the sample) (AOAC, 1998).

2.3.5. Powders flowability

Dynamic angle of repose, loose bulk density, tapped bulk density, Hausner ratio and Carr´s index were determined to analyze powder flow properties.

The dynamic angle of repose is the angle with respect to the horizontal formed by the powder agglomerates. It was evaluated using a cylindrical chamber, which was rotated slightly until the slipping of the powders took place (Solids handling study bench, CEN, United Kingdom). The test was repeated 3 times and the results averaged.

The loose bulk density (ρ_B) of the powders was measured by freely pouring 10 g of the sample in a 100 mL graduated cylinder, without compacting. The value of the tapped bulk density (ρ_T) was also determined by measuring the volume occupied by the powder after hand tapped the cylinder against a flat surface until it reached a constant volume. Five replicates of each assay were performed and then ρ_B and ρ_T were calculated by dividing the sample weight (g) by the measured volume (mL) (Fitzpatrick, 2013).

From the values of ρ_T and ρ_B, the Hausner ratio (HR) and the Carr´s index (CI) were calculated using the following equations:

$$HR=\frac{\rho_T}{\rho_B} \quad (2)$$

$$CI(\%)=\frac{(\rho_T-\rho_B)}{\rho_T}x100 \quad (3)$$

2.4. FTIR analysis

A Fourier transform infrared (FTIR) spectroscopy was performed to identify the main functional groups of the powders, using a spectrometer (Thermo Fisher Scientific Nicolet S10 FT-IR, USA) in the range of 400 to 4000 1/cm. Sixty-four scans were performed per sample by duplicate at a resolution of 4 1/cm. Spectra analysis was performed using the OMNIC™ series software (Thermo Fisher Scientific, USA).

2.5. Total phenolic content, loading capacity and entrapment yield

The Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999) was used to determine the total phenolic content of the powders. Briefly, 2 mL of Na_2CO_3 (2 g/100 mL) were mixed with 200 µL of the sample (in the case of powders, 0.5 g were dissolved in 5 mL of ethanol:water (1:1)) and 200 µL of 1:1 diluted Folin–Ciocalteu reagent (Anedra, Argentina). After 30 min the absorbance was measured at 725 nm in a spectrophotometer (Shimadzu, UV-mini 1240, Japan). Gallic acid (GA) was used as standard. The entrapment yield (EY) was calculated using the following equation:

$$EY(\%) = \frac{L_c}{L_0} x100 \quad (4)$$

where L_0 is total phenol content in propolis extract and L_c is the loading capacity calculated as the total phenolic content of propolis extract loaded on 1 g co-crystallized sample. The results were expressed as mg GA/g powder and mg GA/g propolis.

2.6. Total flavonoid content

The total flavonoid content was determined by reaction with aluminum chloride, according to Popova et al. (2007) with some modifications. The powders (0.5 g) were dissolved in 5 mL of ethanol:water (1:1), and 0.2 mL of PEE was diluted with 10 mL of ethanol:water (1:1). Then, 600 µL of each solution was placed in 25 mL volumetric flasks. A volume of 0.5 mL of aluminum trichloride (Anedra, Argentina) was added to each flask, and the volume was completed with ethanol. After a reaction time of 30 min the absorbance was measured in the spectrophotometer at 425 nm. Quercetin (Q) was used as standard and results were expressed as mg Q/g sample.

2.7. Antioxidant activity of propolis powders

2.7.1. ABTS radical scavenging capacity

The antioxidant capacity test was carried out by the ABTS method, as described by Re et al. (1999), with some modifications. The powder samples (0.5 g) were dissolved in 10 mL of ethanol:water (1:1) and appropriate dilutions were made to fall within the range of the calibration curve. In the case of PEE, 10 µL was diluted with 10 mL of ethanol:water (1:1). One milliliter of the ABTS (Sigma-Aldrich, USA) solution (absorbance: 0.7 ± 0.02, 734 nm) was added to 50 µL of each sample. Absorbance was read at 734 nm after 6 min of the initial mixing. The results were expressed as µmol Trolox equivalent/g sample.

2.7.2. DPPH radical scavenging activity

The antioxidant activity was evaluated by DPPH radical scavenging method (Brand-Williams, Cuvelier, & Berset, 1995). For this purpose, 0.5 g of powder was dissolved in 10 mL of a 50:50 ethanol/water solution. In the case of PEE, 0.2 mL was diluted with 10 mL of ethanol:water (1:1). Then, 100 µL of each solution was mixed with 3.9 mL of 25 mg/L of DPPH• ethanol solution (Sigma-Aldrich, USA). After 30 min in the dark, the absorbance was measured at 517 nm and the results were expressed as µmol Trolox equivalent/g sample.

2.8. Antioxidant stability of propolis powders during storage

The co-crystallized powders were kept at different environmental conditions to evaluate the stability of phenolic and flavonoid compounds. Samples were placed in test tubes closed with a screw cap and stored at 4°C in a refrigerator and at 20°C in darkness and natural light conditions. After 60 d, samples were analysed to determine their total phenolic and total flavonoid contents, as described in sections 2.5 and 2.6.

2.9. Data analysis

Data analysis was performed using Infostat software 2014e version (Di Rienzo et al., 2014). Analysis of variance was performed, and least significant differences were

calculated to compare means at a level of 95% using the Fisher test. A p value < 0.05 was considered significant.

3. Results and discussion

3.1. Physicochemical characteristics of the propolis co-crystallized powders

Fig. 1 shows the macroscopic appearance of the co-crystallized powders with different propolis concentration. As the intended use of the propolis powder is as a food ingredient, colour could define the acceptance of the final product by consumers. Table 2 shows the colour coordinates of the co-crystallized powders and the total colour difference ΔE between samples. The increase in concentration of PEE in the sucrose matrix significantly decreased ($p < 0.05$) the luminosity L* of the samples, whereas, the colour coordinates a* and b* increased ($p < 0.05$) indicating a shift to the red-yellow zone of the CIELab space. ΔE values in Table 2 correlated well with the appearance of CP powders (Fig. 1). López-Córdoba et al. (2014) and Sarabandi et al. (2018) also found a decrease in lightness and a colour enhancement in co-crystallized powders when increased the load of yerba mate and marjoram extracts, respectively, in the sucrose matrix.

Colour of propolis depends on plant source, chemical profile and geographical origin and varies among dark brown, red, green and yellow (Lozina, Peichoto, Acosta, & Granero, 2010; Lopez, Schmidt, Eberlin, & Sawaya, 2014). Revilla, Vivar-Quintana, González-Martín, Escuredo, & Seijo (2017) found a significant correlation among the phenolic composition, the antioxidant activity and the colour of 53 raw propolis samples from Chile and Spain. These authors found that the yellower and paler the colour of the propolis sample, the lower the phenolic content and the antioxidant capacity.

The moisture content of powders can influence particle and bulk properties and physicochemical and biological stability, which consequently will have an impact on handling and processing operations. As shown in Fig. 1, the co-crystallization process

yielded a dried product and only a soft drying at 40°C was needed to obtain an optimal powder with free-flowing particles. Table 2 shows that all CP samples presented low moisture content values (approximately 2%) which are favorable to prevent the spoilage of the products during storage. In addition, propolis itself could contribute to microbiological safety of the powder due to its antimicrobial (Fangio et al., 2019; Kalogeropoulos et al., 2009) and antifungal (Agüero et al., 2014) properties. Other authors (Federzoni et al., 2019; Nik et al., 2019; Sarabandi et al., 2018) reported similar values of water amounts in co-crystallized products.

As shown in Table 2, an increase of PEE concentration led to powders with higher moisture content. Bhandari & Hartel (2002) and previous works in our laboratory (Deladino et al., 2007; López-Córdoba & Navarro, 2018) showed that the active compound (natural extracts, minerals, sugars) included in the sucrose matrix influences on the moisture content of the co-crystallized products.

All CP samples showed high solubility in water (Table 2), which became a good property for propolis powders considering the low solubility of the raw propolis in this solvent. At low concentrations of PEE (CP10 and CP20) there were no significant differences ($p > 0.05$) with the control sample, but at higher concentrations the solubility decreased. As expected by the solubility values observed, the solubilization time increased with higher content of PEE in the powders. The high solubility of sucrose in water and the rapid migration of the solvent through the pores of the agglomerates allow the fast release of the active compound. Probably, the increase of the PEE generates a compacted structure of agglomerates, which leads to a decrease in the solubility.

Sarabandi et al. (2018) also reported that an increase in concentration of marjoram aqueous extract from 3 to 10% w/v reduced the solubility of the co-crystallized powders. Sardar & Singhal (2013) found higher dissolution times for co-crystallized sucrose cubes containing cardamom oleoresin previously emulsified with acacia gum, compared with pure sucrose. These authors attributed this behaviour to differences in

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size and degree of crystallinity of co-crystals since acacia gum increased the compactness of the sucrose cubes. The dissolution of a food powder is a multi-step process involving complex interactions at the solute–solvent interface. Thermodynamic aspect has a crucial role in addition to other typical factors affecting the reconstitution process, such as the powder density, surface area and porosity, fat content and the properties of the dissolving medium (temperature, viscosity, mixing regimes) (Forny, Marabi, & Palzer, 2011).

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3.2. Particle and flow properties of CP powders

Knowledge of the flow behaviour of a powder is useful to predict its handling and caking characteristics during processing, packaging and storage. The size of the particles influences many properties of bulk behaviour of powders, including flowability (Allen, 2003). Fig. 2 shows a monomodal particle size distribution for co-crystallized powders with increasing load of PEE. Significant differences ($p < 0.05$) were found when comparing the total weight of the size fractions. All formulations presented a predominance of particles with size of 500 µm or lower. Except for a small amount of CP10 and the control, particles with size higher than 710 µm did not pass the sieve. The analysis within each size fraction revealed differences depending on the level of PEE used in the formulations (Fig. 2). The particles corresponding to CP10 and CP20 powders were the most abundant with sizes below 500 µm. However, an increase of PEE in the sugar matrix led to a higher weight of 500 µm fraction of particles. A high content of agglomerates of CP40 was still observed in the 710 µm fraction.

As shown in Fig. 2, the content of PEE influenced the particle size of the co-crystallized powders. As PEE increased, the particle size distribution shifted to higher sizes. Thus, at low concentrations of the active compound the matrix structure was determined by the sucrose agglomerate, but as the volume of propolis extract increased, the encapsulated compound also had an impact.

Flowability of co-crystallized powders was characterized through the dynamic angle of repose (Table 3). Higher propolis content in the sucrose matrix led to increasing values of angles of repose, with significant differences ($p < 0.05$) for CP30 and CP40 samples. According to de Jong, Hoffmann, & Finkers (1999), values between 30 and 45º indicate that the material can flow freely, the range 45-60º corresponds to fairly free-flowing powders and values higher than 60° are indicative of cohesive materials. Thus, formulations with low propolis content (CP10 and CP20) are free-flowing powders, CP30 is a fairly free-flowing powder and CP40 has difficulties to flow due to its cohesiveness. The latter could be because CP40 formulation presented high moisture content (Table 2), which could make it more difficult for particles to slip over others.

Several authors also obtained co-crystallized powders with good flow properties when encapsulating extracts derived from plants (López-Córdoba et al., 2014), herbs (Sarabandi et al., 2018), seeds (Nik et al., 2019), fruits (Karangutkar & Ananthanarayan, 2020), among others. In the present work, the co-crystallization process was able to produce powders with high fluidity even at higher propolis concentration.

Table 3 shows no significant differences ($p > 0.05$) between bulk density values for the control and CP10, but for higher concentrations of PEE this parameter decreased. This could be attributed to the fact that a higher initial concentration of sucrose (CP10) leads to a smaller size of agglomerates (Fig. 2), which are better packaged, with less air between particles and a greater weight per unit of volume. Deladino et al. (2007) analysed co-crystallized systems with yerba mate extract and different salts and obtained bulk density values between 0.65 and 0.72 g/cm^3, similar to CP40 bulk density value. Federzoni et al. (2019) also found a similar result (0.789 g/cm^3) for paprika oleoresin co-crystallized powders.

As expected, tapped density of samples also decreased with propolis concentration (Table 3), values ranging from 1.00 g/cm^3 (control) to 0.77 g/cm^3 (CP40). Table 3

shows an HR value significantly higher for CP40 compared to the rest of the propolis co-crystallized powders. The samples could be considered powders with good (CP10 and CP20) and fair (CP30 and CP40) characteristics, since HR in the range 1.0-1.11 corresponds to excellent, 1.12-1.18 good and 1.19-1.25 fair flow powder character (Fitzpatrick, 2013). The Hausner ratio correlates with the presence of attractive forces and friction in the powder bed (Hayes, 1987). When PEE increased, the changes observed in the properties of the sugar matrix, like particle size (Fig. 2) and tapped and bulk densities (Table 3), confirmed the increase in the values of angle of repose and correlated well with Hausner ratio. Therefore, HR was a useful quality parameter to evaluate the flowability of propolis co-crystallized powders.

CI is a useful parameter to evaluate the powder compressibility, which is a property defined as the ability of a material to reduce volume under pressure (Barbosa-Cánovas, Ortega-Rivas, Juliano, & Yan, 2005). Lower CI values are indicative of better compressibility. Results in Table 3 showed that PEE decreased the compressibility of the co-crystallized powders compared to the control. The same behaviour was found in co-crystallized powders by López-Córdoba et al. (2016) and Nik et al. (2019). CI values up to 10% are considered excellent (control sample), between 10 and 15% are good (CP10, CP20 and CP30) and between 16 and 20% are poor (CP40) (USP 30-NF 25, 2007). Compressibility results were in agreement with the repose angle values (Table 3), indicating that levels of propolis below 56.6 mg/g (Table 1) led to co-crystallized powders with good flowability and compressibility.

3.3. Identification of functional groups by FTIR spectroscopy

FTIR analysis allowed identifying the absorbance bands of the functional groups present in the PEE and CP powders (Fig. 3). The co-crystallized samples showed signals at the following frequencies: 3328, 3560, 2941, 1321, 1048 and 941 1/cm related to the stretching vibration of the OH groups, symmetrical and asymmetrical stretching of CH_2, deformation of OH groups, and stretching of the CO bond,

406 respectively. The fingerprint region (700 – 1700 1/cm) contains much more valuable
407 information than the broad bands between 2900 and 3600 1/cm. The characteristic
408 bands of the sucrose molecule (Brizuela et al., 2012) were found in the control and CP
409 samples, suggesting that conformational changes of the sugar did not take place
410 during the co-crystallization process (López-Córdoba et al., 2015). Sucrose bonds in
411 co-crystallized products were similar to the control sample, indicating that no reaction
412 occurred between the active compound and sucrose. Similar spectroscopic
413 characteristics were also observed by Sarabandi et al. (2018) in co-crystallized
414 marjoram extract.

415 The propolis extract (10% w/v) was dissolved in ethanol, therefore, the FTIR signals
416 at 1045 and 1087 1/cm (Fig. 3) should be assigned to this solvent (Coldea et al., 2013).
417 The fingerprint region of the PEE spectrum showed signals corresponding to the
418 different modes of vibration of flavonoids, aromatics rings and secondary alcohols
419 associated to these structures. The polyphenols bands are found in the regions
420 between 1040 and 1150 1/cm due to the C-O bond vibration, and between 1180 and
421 1270 1/cm attributed to the stretching of the phenolic OH. The region between 1150 to
422 1350 1/cm is related to the CH_3 symmetrical vibrations, and the signal at 1640 1/cm,
423 typical from the aromatic systems, corresponds to stretching vibrations of C=C and
424 C=O of flavonoids, and asymmetric bending vibration of N-H due to aminoacids (Wu,
425 Sun, Zhao, Li, & Zhou, 2008, Fangio et al., 2019). The peak at 1645 1/cm found in the
426 PEE spectrum (Fig. 3) was also observed (as a very weak signal) with an incremental
427 intensity from CP10 to CP40 spectra, indicating the presence of propolis in the
428 powders. The rest of the bands could not be distinguished due to the propolis
429 components were in low concentration or were overlapped by the peaks of the sucrose.
430

431 **3.4. Antioxidant activity and stability during storage of CP powders**

432 Fig. 4 shows that a higher content of PEE in the co-crystallized powders led to a
433 significant ($p < 0.05$) increase in the polyphenol and flavonoid content (Fig. 4a and 4b).

PEE had a total polyphenol content of 307.9 mg GA/g and a flavonoid content of 58.8 mg Q/g. Fangio et al. (2019) analysed propolis from Buenos Aires province (the same as the material used in this work) and found values of polyphenols ranging from 189 to 417 mg AG/g and flavonoids ranging from 46 to 215 mg Q/g. The antioxidant activity measured by DPPH and ABTS methods also followed the same behaviour for all samples (Fig. 4c). This is an expected result since antioxidant activity is highly correlated to the phenolic content of propolis, foods and plants (Kumazawa, Hamasaka, & Nakayama, 2004; Jacobo-Velázquez & Cisneros-Zeballos, 2009). Thus, the increasing amount of phenolic compounds loaded in the powder structure led to an equivalent increase of antioxidant activity.

For each formulation, the expected values for polyphenols and flavonoids after the co-crystallization process were close to the loaded values in the sucrose matrix (Fig. 4a and 4b), indicating high entrapment yield for propolis extract. Minimum retention values of bioactive compounds in the matrix were 84% for flavonoids (91% average) and 78% for phenolics (95% average). López-Córdoba et al. (2014) and Sarabandi et al. (2018) reported values of entrapment yield of 84% and 85% of total phenolic content, respectively. Federzoni et al. (2019) found a higher value of retention (95%) of β-carotene from paprika oleoresin in the agglomerated matrix. The slight decrease in the polyphenol and flavonoid content could be due to degradation, or even destruction, of these antioxidant compounds by heating during the co-crystallization process. In spite of those decreasing values, the high entrapment yields found in all formulations indicated that propolis co-crystallized powders could be considered as bioactive matrixes.

The stability of propolis antioxidant compounds during storage is an important aspect to consider for the use of co-crystallized powders as food ingredients. Fig. 5 shows the effect of temperature (4°C) and illumination conditions (light and darkness) in polyphenols and flavonoids after 60 d of storage of CP powders. In general, after 60 d of storage a higher stability of propolis phenolic compounds was found in the

462 samples with high PEE content (CP30 and CP40), except CP30 that was affected by
463 darkness condition. In the case of the co-crystallized powders with lower PEE
464 concentration, a significantly ($p < 0.05$) decrease in their phenolic content was
465 observed under light and darkness for CP10 and at the three conditions assayed for
466 CP20 (Fig. 5a). Sarabandi et al. (2018) also found that the co-crystallized marjoram
467 extracts loaded with the highest extract concentration were more stable during storage
468 at different conditions. It is worth noting that these authors loaded the same volume of
469 different marjoram extract concentrations (3, 5, 10% w/v) and in the present work
470 different volumes of 10% w/v propolis extract were used. Probably, the agglomerates
471 could hold higher content of active compound in the void spaces and they would be
472 more protected from the environment conditions.

473 Darkness storage of CP powders decreased significantly ($p < 0.05$) the polyphenol
474 content (except for CP40), while exposure to light had the same effect on the CP10
475 and CP20 samples (Fig. 5a). Only phenolic compounds in CP20 powders were
476 affected by the low temperature, decreasing their content. After both co-crystallization
477 process and storage time, percentage losses of polyphenols of the loaded extract
478 ranged from 16 to 31%. Even though, the encapsulation technique was able to
479 maintain a high level of propolis polyphenols present in the ethanolic extract. Storage
480 conditions like artificial light and relative humidity of 75% caused deterioration of total
481 phenolic content in co-crystallized plant extracts (Sarabandi et al., 2018). However, low
482 storage temperatures (10°C) maintained the percentage of propolis phenolic
483 compounds constant over 180 d of storage (Nori et al., 2011). This was attributed to
484 the greater mobility of compounds at higher storage temperatures and, thus, they are
485 more subject to degradation reactions.

486 Fig. 5b shows that flavonoid content decreased by refrigerated storage in all CP
487 samples, except for CP10 which maintained its value. Percentage losses of flavonoids
488 of the loaded PEE ranged from 7 to 35% after both co-crystallization process and
489 storage time. Unlike phenolic compounds, flavonoids were retained or even increased

after storage in light and darkness conditions. Fig. 5c compares the ratio (total flavonoids/ total phenolics) in every CP sample with this ratio in the PEE. Refrigeration was the only condition that decreased this ratio in all samples, respect to the PEE, after 60 days of storage. Flavonoid content in propolis samples can be considered a good marker of their quality (Gardana, Scaglianti, Pietta, & Simonetti, 2007), thus, propolis co-crystallized powders could maintain the propolis quality under storage in light or darkness conditions.

4. Conclusions

Propolis ethanolic extract was transformed into a free-alcohol powder through a co-crystallization process in a sucrose matrix. Different levels of the propolis extract were loaded into the co-crystallized sugar obtaining powders with fine particle size, low moisture content and high solubility in water. These were good characteristics of the co-crystallized powders, especially the latter considering the low solubility of the propolis in this solvent.

Co-crystallized powders with low propolis contents had good flowability; however, the increase in the extract concentration affected the flow properties of the powders. Bioactive compounds of propolis, like polyphenols and flavonoids, were encapsulated in the co-crystallized powder with high efficiency, contributing to the antioxidant activity in the sucrose matrix. Even though these compounds were mostly retained during storage of powders, flavonoids were more affected by refrigeration condition and polyphenols by light and darkness exposure. Regarding composition, powders with high content of propolis extract were more stable to storage conditions.

The propolis co-crystallized powder may open new applications as a food ingredient with functional activity and the presence of sucrose would mask the astringent taste of propolis. The good physicochemical and technological properties of these powders would be an advantage during processing and storage of the final products.

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Figure captions

Figure 1. Co-crystallized powders with different contents of propolis extract.

Figure 2. Size distribution of ground co-crystallized powders. Control (□), (■): CP10, (■): CP20, (■): CP30, (■): CP40. Values with the same letter (capital letters for size comparison, lowercase letters for composition comparison) are not significantly different ($p > 0.05$). ND: not detectable.

Figure 3. FTIR spectra of propolis extract and co-crystallized powders with different propolis contents.

Figure 4. Polyphenol (a) and flavonoid (b) contents and antioxidant activity by DPPH (■) and ABTS (■) (c) of propolis co-crystallized powders. White and black striped portions of the columns (Fig. a, b) indicate the loaded and measured values, respectively. GA: gallic acid, Q: quercetin.

Figure 5. Effect of storage conditions on the phenolic (a) and flavonoid (b) contents and on the total flavonoids / total phenolics ratio (c) of propolis co-crystallized powders. Not stored (□), refrigerated (■), light (■) and darkness (■). Dashed line corresponds to the ratio in PEE. GA: gallic acid, Q: quercetin. Values with the same letter are not significantly different ($p > 0.05$).

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Table 1. Formulations used to obtain co-crystallized propolis (CP) powders with different propolis content. PEE: propolis ethanolic extract.

Ingredient	Control	CP10	CP20	CP30	CP40
Sucrose (g)	50	50	50	50	50
PEE (mL)	0	10	20	30	40
Ethanol (mL)	10	0	0	0	0
Distilled water (mL)	10	10	10	10	10
Propolis content (mg propolis/g powder)	0	19.6	38.5	56.6	74.1

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Table 2. Physicochemical properties (colour, moisture content, solubility) of co-crystallized powders with different propolis contents.

Sample	L*	a*	b*	ΔE	Moisture (g/100 g)	Solubility (%)	Solubilization time (s)
Control	95.52±0.28^{e}	-0.447±0.01^{a}	4.07±0.10^{a}		0.53±0.04^{a}	98.93±0.02^{c}	38.00±0.00^{a}
CP10	82.26±0.88^{d}	3.99±0.06^{b}	21.00±0.09^{b}	21.96±0.67^{a}	0.03±0.04^{a}	98.64±1.06^{c}	41.12±2.67^{ab}
CP20	71.26±1.11^{c}	7.43±0.17^{c}	24.95±0.47^{c}	31.32±2.98^{b}	0.66±0.01^{a}	98.83±0.00^{c}	41.50±0.71^{b}
CP30	68.35±0.89^{b}	8.80±0.18^{d}	27.53±0.37^{d}	37.07±1.09^{c}	1.24±0.04^{b}	97.18±0.33^{b}	45.50±0.71^{c}
CP40	62.00±1.00^{a}	11.03±0.15^{e}	30.10±0.20^{e}	43.96±0.95^{d}	2.09±0.06^{c}	95.76±0.32^{a}	47.00±0.00^{c}

Values with the same letter in the same column are not significantly different ($p > 0.05$).

Values are mean ± standard deviation of at least three replicates.

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Table 3. Flow properties (dynamic angle of repose, Hausner ratio, Carr´s index) and bulk and tapped density of co-crystallized powders with different propolis content.

Sample	Dynamic angle of repose (º)	HR	CI (%)	Bulk density (g/cm^3)	Tapped density (g/cm^3)
Control	41.17±2.02^{a}	1.05±0.07^{a}	9.09±0.00^{a}	0.95±0.06^{c}	1.00±0.00^{b}
CP10	42.25±1.06^{a,b}	1.15±0.07^{a}	12.67±1.06^{b}	0.87±0.05^{c}	1.00±0.00^{b}
CP20	43.75±1.77^{a,b}	1.16±0.03^{a}	13.94±2.04^{b}	0.80±0.05^{b}	0.93±0.03^{b}
CP30	48.25±3.89^{b}	1.19±0.07^{a}	15.62±1.11^{b}	0.78±0.02^{b}	0.93±0.03^{b}
CP40	70.50±3.03^{c}	1.22±0.03^{b}	18.14±1.26^{c}	0.63±0.01^{a}	0.77±0.00^{a}

Values with the same letter in the same column are not significantly different ($p > 0.05$).

Values are mean ± standard deviation of at least three replicates.

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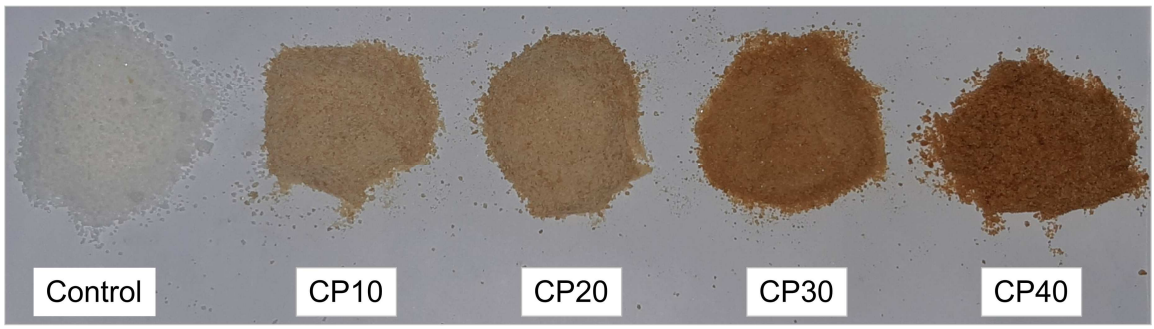

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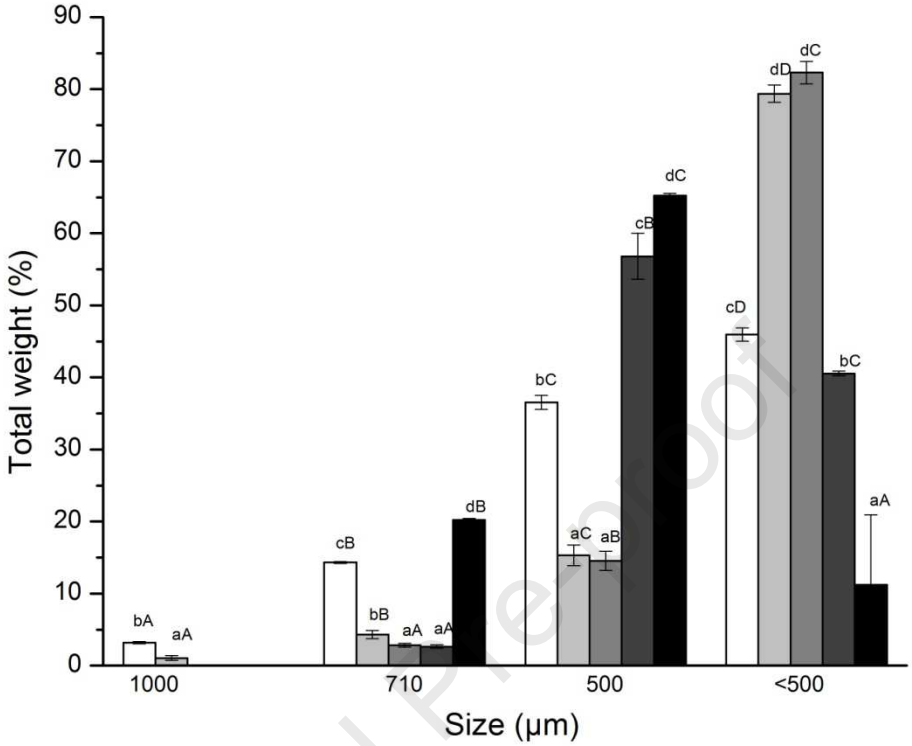

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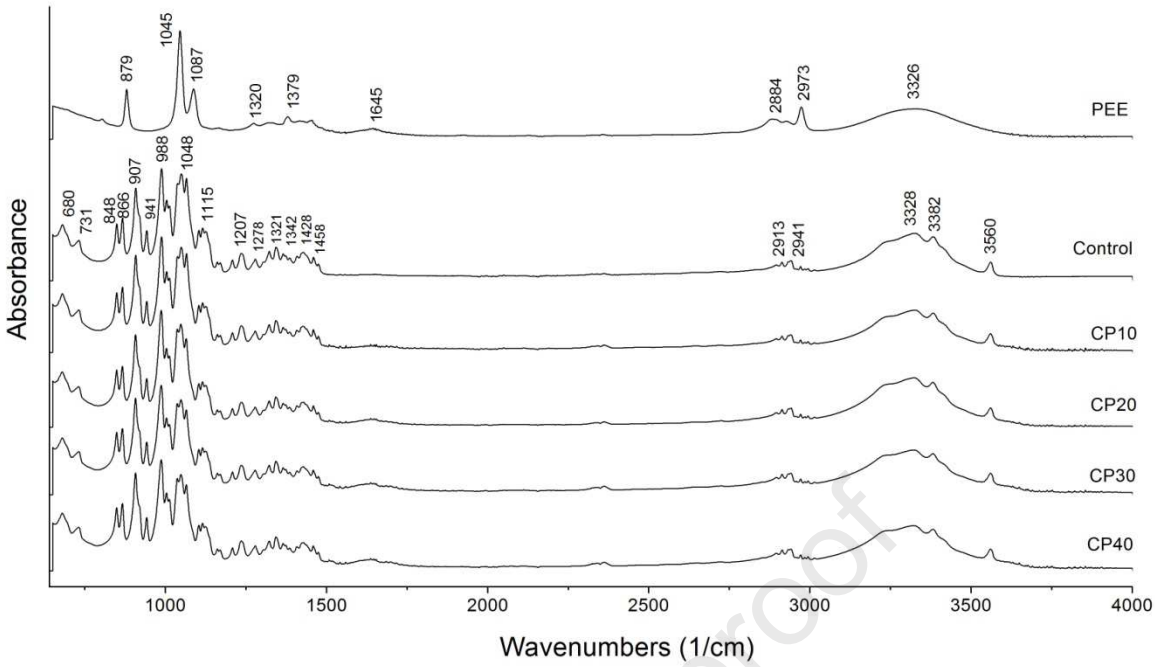

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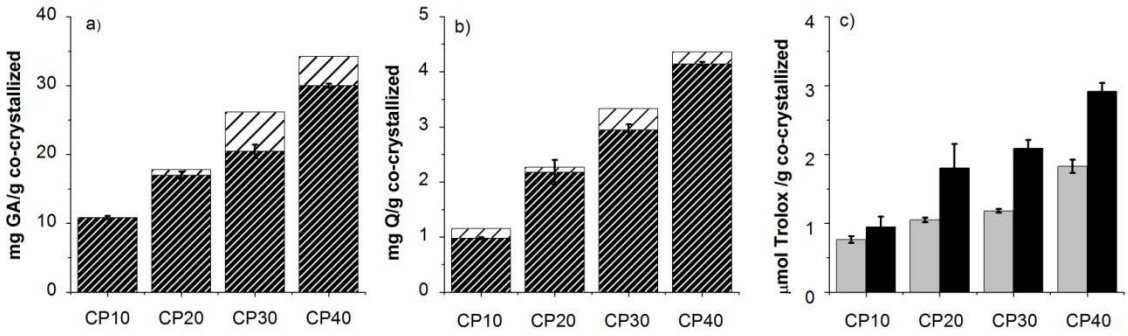

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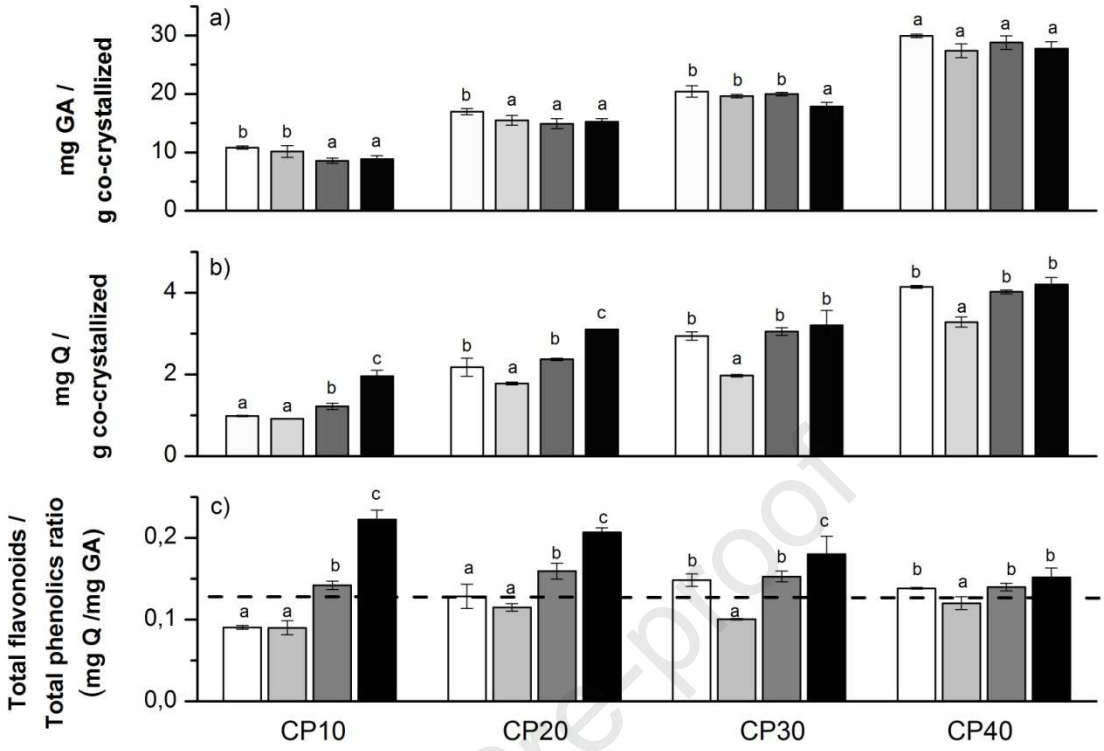

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Highlights

Free-alcohol propolis powder was obtained by co-crystallization in a sucrose matrix.

Propolis powders had good technological properties.

Powder flowability was affected by a high load of propolis extract.

High content of antioxidant compounds of propolis was retained after powder storage.

Bioactive co-crystallized propolis powders may be used as food ingredients.

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Declaration of interests

X The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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