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Design of a microencapsulated propolis extract with controlled release by spray drying †

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Abstract: The aim of this work was to optimize the microencapsulation of a propolis extract (PE) with inulin (IN) by spray drying to obtain an ingredient (PE-IN) with properties of control release in gastrointestinal tract. The phenolic compounds from propolis were extracted by 2 cycles of maceration with ethanol:water (70:30, v/v) in agitation during 24 hours at room temperature. PE was comprehensively characterized by HPLC-ESI-QTOF-MS/MS and the microencapsulation of PE/IN by spray drying was optimized by response surface using a central composite. The optimal conditions for PE-IN microencapsulation were an inlet air temperature of 112.65°C and a PE/IN ratio of a 1:4.315, showing a yield of 78.4%, 71.7% of encapsulation efficiency and 95% of recovery. Finally, the bioaccessibility was measured by INFOGEST method, showing changes in the release profile of phenolic compounds in the PE-IN microparticles.

Keywords: Propolis; bioactive compounds; microencapsulation; spray drying; bioaccessibility

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1. Introduction

Propolis is a natural product that bees make from wax, salivary secretions and resinous material that bees collect from flowers and leaf buds of certain plants [1-2]. Bees use propolis to build and repair their hives and to disinfect them, since propolis is a good thermal insulator and it has antimicrobial properties as well [3-4].

The composition of propolis consists mainly of resins and balms (50-55%), wax (25-35%), volatile oils (10%), pollen (%), organic and mineral substances (5%) [5]. In addition, propolis contains a wide variety of bioactive compounds such as flavonoids, phenolic acids derivatives and other phenolic compounds, as well as terpenes and terpenoids [6-8]. All these bioactive compounds confer several beneficial properties to propolis, such as antioxidant, anti-inflammatory, antifungal, among other [8].

However, some of these compounds with bioactive properties are easily degraded, for example, by light [9]. To prevent its degradation, the spray drying technique can be used. This technique allows to cover and protect bioactive compounds in a polymer matrix and release these compounds in different parts of the gastrointestinal tract [10]. For this, polymers with release properties in the colon such as inulin are used. This polysaccharide can act as substrates for the bacterial microbiota inhabiting the large

intestine. The degradation of polysaccharide matrix molecules depends mainly on the hydrolysis of the glycosidic bonds between the molecules and the subsequent release of the bioactive components [11]. In this way, the bioaccessibility of the bioactive compounds present in propolis is increased.

2. Materials and Methods

2.1. Samples

Propolis was provided by "Apícola Valle del Maitena-Apicultura El Cañuelo" (Güéjar Sierra, Granada, Spain). A propolis sample was pretreated with hexane for dewaxing and then, the phenolic compounds were extracted by 2 cycles of maceration with ethanol:water (70:30, v/v) in agitation during 24 hours at room temperature. The propolis extract (PE) was stored in falcon tubes at 20°C in dark conditions until its microencapsulation.

2.2. Characterization of the propolis extract (PE) by HPLC-ESI-QTOF-MS/MS.

The HPLC-MS analyses were performed on an Agilent 1260 instrument (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 6540 Ultra High Definition (UHD) Accurate-Mass Q-TOF mass spectrometer equipped with a Jet Stream dual ESI interface operating in negative ion mode. The analytical column used was an Agilent Zorbax Eclipse Plus C18 (150 mm x 4.6 mm, 1.8 μm).

The mobile phases consisted of water with 0.1% formic acid (A) and acetonitrile (B) with a gradient elution according to the following multistep profile: 0 min, 5% Solvent B; 5 min, 30% Solvent B; 35 min, 95% Solvent B; 40 min, 5% Solvent B; 45 min, 5% Solvent B. The flow rate was 0.5 mL/min, the temperature of the column was maintained at 25 °C and the injection volume was 5 μL. Detection was performed in a mass range of 50–1700 m/z with the continuous infusion of the reference ions m/z 112.985587 (trifluoroacetate anion) and 1033.988109 (adduct of hexakis (1H,1H, 3H-tetrafluoropropoxy) phosphazine or HP-921) to correct each mass spectrum.

2.3. Encapsulation of phenolic compounds from PE by spray drying.

Microparticles of Propolis extract (PE) were prepared with Inulin (IN), obtain the PE-IN ingredient, as follows: 50 g of feed solution was prepared by weighing 5 g of inulin which was dissolved in water (37.7-43.75 g) at 70°C and then cooled to 30°C and mixed with EP (1.16-7.3 g) with constant stirring. The resulting solutions were homogenized at 15,000 rpm for 5 min with an Ultra Turrax T25 (IKA, German) and fed into a 4M8-TriX spray-dryer (ProCepT, Belgium).

2.4. Experimental design.

The microencapsulation experiments for PE-IN were performed using a central composite design with 12 runs (4 experimental points, 4 axial points, and 4 central points). The air inlet temperature (112.65–197.35 °C) and the PE/IN ratio (1:0.6849–1:4.321) were evaluated as independent variables according to Yield of drying (Y), Encapsulation Efficiency (EE) and Recovery (Rec) of Total Phenolic Content (TPC) and Antioxidant capacity measured by FRAP Method (FRAP).

Response surface methodology (RSM) was applied to determine the optimal conditions for PE-IN systems by multiple response optimization using the desirability function (DF) where the response variables were maximized.

2.5. Characterization of the microparticles (PE-IN).

PE-IN microparticles were characterized according to Surface and Total content of TPC by Folin-Ciocalteu method [12] and FRAP [13], for which differentiated extractions were carried out for the surface and total in the following way.

2.5.1. Surface content.

Microparticles (200 mg) were mixed gently in 1.5 ml ethanol:methanol (1:1 v/v) solution and centrifuged at 1,000 RPM for 1 min al 4°C. TPC and FRAP were quantified according to standard methods previously reported [12, 13].

2.5.2. Total content.

The coating material structure of the microparticles was completely destructed by the following procedure: microparticles (100 mg) were dispersed in 0.75 ml of water:ethanol:methanol (2:1:1, v/v/v), stirred using a vortex mixer for 1 min, ultrasonicated twice for 20 min, and then centrifuged at 5,000 RPM for 5 min at 4°C. The supernatant was collected and the precipitate dispersed in 0.75 ml of water:ethanol:methanol (2:1:1, v/v/v) repeating the above procedure. TPC and FRAP were quantified.

The encapsulation efficiency (EE) was calculated according to Eq. (1).

$$EE(\%) = \frac{Experimental\ total\ content - superficial\ content}{Experimental\ total\ content} x\ 100 \quad (1)$$

The recovery (Rec) was calculated according to Eq. (2)

$$Rec(\%) = \frac{Experimental\ total\ content\ in\ the\ powder}{Experimental\ total\ content\ in\ the\ feed\ solution} x\ 100 \quad (2)$$

And yield (Y) was calculated according to Eq. (3)

$$Y(\%) = \frac{Powder\ after\ spray\ drying\ (g)}{Solids\ in\ the\ feed\ solution\ (g)} x\ 100 \quad (3)$$

2.6. Bioaccessibility of phenolic compounds from PE and PE-IN.

The bioaccessibility of different phenolic compounds from PE and PE-IN was measured by INFOGEST method [14], it was sampled at the end of simulated gastric digestion (SGD) and at the end of simulated intestinal digestion (SID) and quantified by HPLC-ESI-QTOF-MS/MS according to the methodology 2.2.

3. Results and Discussion

3.1. PE obtention

A propolis sample was dewaxed with hexane, obtaining a propolis fine powder which facilitated the subsequent extraction process. By this procedure, it was obtained a 71.6% of matter from the starting propolis, value that corresponds to the wax content of propolis previously found (25-35%) [15]. Then, the propolis extraction was carried out with (ethanol:water,70:30 v/v) with a extraction yield of 67%.

3.2. Characterization of the PE by HPLC-MS

The propolis extract was reconstituted at a concentration of 5 $mg{\cdot}mL^{-1}$ in the extraction solvent and it was analyzed by HPLC-MS, obtaining the chromatogram shown in Figure 1. The main compounds were automatically detected by a molecular features extraction algorithm and the found peaks were filtered with a volume threshold at 0.3% with respect to the main peak, being detected a total of 66 compounds. The detected compounds by this procedure were tentatively identified whenever possible by interpretation of their MS and MS/MS spectra obtained by QTOF-MS/MS combined with the data provided by databases and the literature.

In this way, 58 compounds could be identified in the propolis extract, corresponding the major compounds to phenolic acid derivatives, such as caffeic acid and coumaric acid

derivatives, as well as flavonoids, such as pinobanksin, chrysin, pinocembrin and their derivatives. The two major compounds correspond to prenyl caffeate isomers (8.97% and 6.59% of peak relative volume), followed by pinobanksin acetate and pinobanksin (5.71% and 3.32%, respectively). It should be noted that the characterization of the extract was very similar to that previously described in the scientific literature, since the compounds identified as majority coincide [16-20].

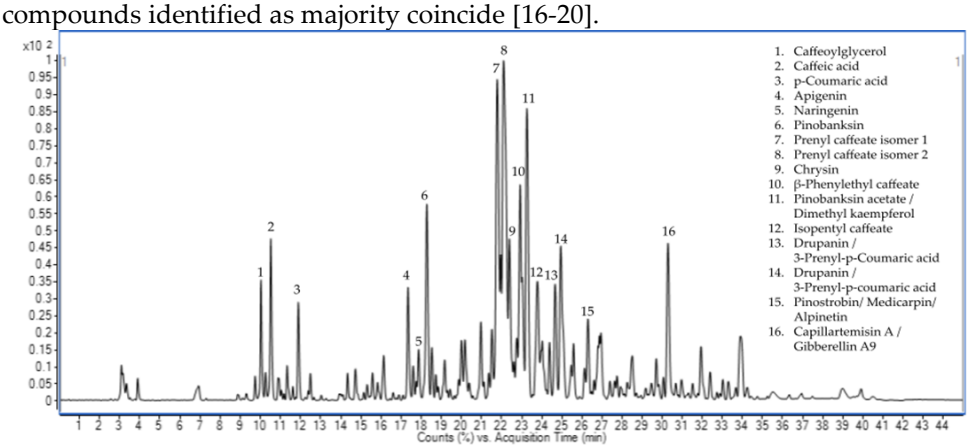

Figure 1. Base Peak Chromatogram (BPC) of the PE at a concentration of 5 mg·mL^{-1}.

3.3. Encapsulation of phenolic compounds from PE by spray drying.

A central composite design for the microencapsulation of PE was carried out to evaluate the effect of the drying process (air inlet temperature) and the formulation (PE/IN ratio) on response variables. Table 1 shows the experimental conditions, response variables and ANOVA for response variables used in PE-IN microencapsulation.

The results showed that yield varied between 69.2 and 79.1% and only its linear form of PE/IN ratio was significant. For EE´s the values were 40.0-70.2% for TPC and 14.5-74.8% for FRAP. The linear and quadratic forms of temperature, the linear form of PE/IN ratio and cross-product form were significant for EE TPC. Instead, for EE FRAP all forms were non-significant.

In relation with the Recovery, the values were 35.6-91.0% for TPC and its quadratic forms of temperature, the linear and quadratic form of PE/IN ratio and cross-product form were significant. However, the FRAP Recovery showed values between 81.1 and 123.1% and all forms were non-significant. Values greater than 100% may be due to an effect of drying conditions; high temperatures may produce transformations in the structure of phenolic compounds which could be associated with a greater antioxidant capacity.

Table 1. Experimental design for the microencapsulation of PE by spray drying, response variables, ANOVA for yield, encapsulation efficiency (EE) and Recovery (Rec) of PE-IN microparticles.

Treatment	Temperature (°C)	PE/IN ratio	Y (%)	EE TPC (%)	EE FRAP (%)	Rec TPC (%)	Rec FRAP (%)
T_1	155	1:2.5	75.2	67.3	62.8	65.6	86.1
T_2	155	1:2.5	73.3	63.0	56.9	62.6	81.1
T_3	190	1:4	73.4	62.1	62.8	51.3	84.2
T_4	155	1:2.5	74.4	64.7	60.4	73.3	89.2
T_5	155	1:0.685	69.2	56.1	61.9	60.2	99.4
T_6	120	1:1	72.8	40.0	64.5	35.6	123.8
T_7	197.35	1:2.5	77.0	67.4	71.2	55.6	106.1
T_8	155	1:4.315	79.1	62.3	69.1	82.8	111.4
T_9	155	1:2.5	75.5	68.6	74.8	59.2	104.5
T_{10}	190	1:1	71.2	70.2	14.5	51.3	105.4
T_{11}	120	1:4	78.5	68.9	64.9	91.0	104.8

T_{12}	112.65	1:2.5	71.3	64.0	65.6	55.6	88.3
Forms				*p-value*			
Temperature			0.9772	0.0194*	0.2473	0.2047	0.6531
PE/IN ratio			0.0239*	0.0173*	0.1556	0.0027*	0.5129
Temperature^2			0.8062	0.7705	0.6296	0.0298*	0.5360
PE/IN ratio^2			0.5125	0.0014*	0.1249	0.0050*	0.9401
Temperature × PE/IN ratio			0.8062	0.0214*	0.4472	0.5247	0.1887

^{2} = Quadratic functions; *Significant effect.

The multiple optimization considering all response variables with significant effects was evaluated (Desirability Function). Figure 2 shows the surface response graphic. The optimal conditions for the PE-IN microencapsulation by spray drying were 112.65°C of inlet temperature and a PE/IN ratio of 1:4.315. In the graph of the response surface, the optimum values are located at the red zone with 0.9798 of desirability. Showing a yield of 78.4%, in addition to 71.7% and 95.0% of TPC EE and TPC Recovery of total phenolic compounds, respectively.

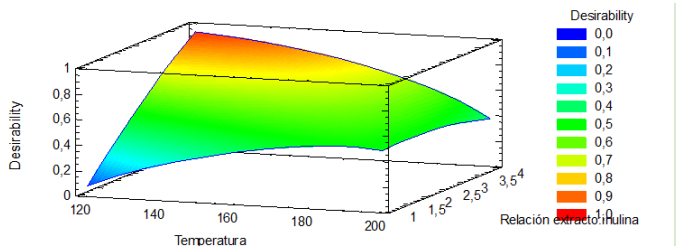

Figure 2. Desirability function overlay surfaces plots of PE-IN microencapsulation by spray drying.

3.4. Bioaccessibility of phenolic compounds from PE and PE-IN.

Table 2. shows the bioaccessibility values of the major phenolic compounds of the propolis extract.

Table 2. Bioaccessibility (%) of phenolic compound from PE and PE-IN.

Compounds	**PE**		**PE-IN**	
	SGD	**SID**	**SGD**	**SID**
Prenyl caffeate isomer 1	16,1 ± 0,5	11,1 ± 0,8	13,4 ± 0,2	22,6 ± 0,5
Prenyl caffeate isomer 2	10,7 ± 0,3	6,0 ± 0,4	11,0 ± 0,3	17,6 ± 0,6
Pinocembrin	15,3 ± 0,1	25,1 ± 3,2	9,8 ± 0,2	39,3 ± 1,1
Pinobanksin	93,4 ± 3,1	83,8 ± 10,8	49,5 ± 1,2	93,2 ± 2,2
Pinobanksin acetate	11,0 ± 0,4	20,58 ± 2,1	9,0 ± 0,1	46,2 ± 1,6

average ± standard deviation (n=3).

When comparing the bioaccessibility of the phenolic compounds present in PE and PE-IN, it is observed that in PE-IN gastric bioaccessibility decreases and intestinal bioaccessibility increases, showing a change in the release profile of the different phenolic compounds studied.

4. Conclusions

The encapsulation of PE by spray drying with inulin is a good alternative to design microparticles with targeted delivery, achieving good yields, high EE% and Rec% and modifying the release profile of phenolic compounds present in the propolis extract, allowing its use in the development of functional and/or nutraceutical ingredients.

Contributing to the development of beekeeping production and the revaluation of this resource.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Poster: Design of a microencapsulated propolis extract with controlled release by spray drying.

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