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Antioxidant, antimicrobial and anti-inflammatory activities of ling-heather honey powder obtained by different methods with several carriers

Sandra María Osés, Leire Cantero, Guillermo Puertas, Miguel Ángel Fernández-Muiño **, María Teresa Sancho *

Department of Biotechnology and Food Science, Universidad de Burgos (University of Burgos), Plaza Misael Bañuelos s/n, 09001, Burgos, Spain

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ABSTRACT

Honey powder is being increasingly used in food, cosmetic and pharmaceutical industry, because it avoids inconveniences of raw honey, such as high viscosity, stickiness and formation of sugar crystals. It is of paramount importance to know if honey powders keep the features of raw honeys. This is the first study, in which total phenolics, total flavonoids, and biological properties of ling-heather (*Calluna vulgaris* (L.) Hull) honey powders obtained by different drying methods (spray drying, vacuum drying and freeze drying), using different carriers (Arabic gum, whey protein isolate and maltodextrin) were assessed. Results showed that all the drying procedures and carriers retained the honey phenolics in the honey powders. Honey powders' antioxidant activities against different free radicals ($ABTS^{\bullet+}$, $ROO^{\bullet}$, $^{\bullet}OH$ and $O_2^{\bullet-}$) were higher than those of the raw honeys, while honey powders' anti-inflammatory and antimicrobial activities against several microorganisms (*Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli*) were lower. Concentration of honey in the powder and type of carrier used for dehydration were the key factors for the quality of honey powders. However, the drying procedure did not strongly influence the parameters and properties studied.

1. Introduction

Nowadays, there is a high consumer demand for foods that promote health benefits, such as honey. Honey is a natural foodstuff produced by bees that has been used since ancient times as a traditional medicine for wounds, microbial infections, and burns, among other conditions (Bogdanov, 2016). Many research papers highlight a variety of potentially functional properties of honeys (Alevia et al., 2021; Molan, 1992; Seraglio et al., 2019; Álvarez-Suárez, Tulipani, Romandini, Bertoli, & Battino, 2010), mainly attributed to phenolic compounds, organic acids, amino acid, enzymes and Maillard reaction products (Álvarez-Suárez et al., 2010; Bogdanov, Jurendic, Sieber, & Gallmann, 2008). Honey is increasingly used as sugar's substitute in candies, confectionery and bakery products, being also added to different foods, such as sausages, beef patties, fish, fruits and vegetables, in order to extend their shelf life, and/or to enhance their bioactive properties (Chen, Mehta, Berenbaum, Zangerl, & Engeseth, 2000; Hakim, Tjahjaningsih, & Sudarno, 2019; Johnston, Sepe, Miano, Brannan, & Alderton, 2005; Półtorak et al., 2018). Nevertheless, the uses of honey are limited because of its high viscosity, stickiness and the formation of glucose crystals, causing handling problems (Suhag, Nayik, & Nanda, 2016). To solve them, recent studies were focused on making honey powders appealing to the consumers, with optimal physicochemical properties that could be easily stored, transported and mixed with other food products, such as bread and turkey breast meat (Antony, Rieck, & Dawson, 2000; Ram, 2011; Rivero, Archaina, Busquet, Baldi-Coronel, & Busch, 2021; Samborska, 2019; Suhag, Nayik, Karabagias, & Nanda, 2021). However, powdered honey is difficult to make, because of the growing stickiness produced as a consequence of the drying procedure. The stickiness is due to the chemical composition of honey, mainly to the low molecular weight sugars, such as glucose and fructose, which exhibit low glass transition temperatures (T_g) (31 and 5 °C, respectively) (Jayasundera, Adhikari, Aldred, & Ghandi, 2009). The T_g of honey powders vary between −27 and −51 °C (Osés et al., 2021; Sramek, Woerz, Horn, Weiss, & Kohlus, 2016). Honey powder is commonly obtained by different procedures, being spray drying the most researched (Jedlińska et al., 2019; Samborska, 2019; Shi, Fang, & Bhandari, 2013; Suhag & Nanda, 2015). Other procedures, such as vacuum drying (Nurhadi, Andoyo, Mahani, & Indiarto, 2012; Sahu, 2008), freeze drying (Rivero et al.,

* Corresponding author.
** Corresponding author.
E-mail addresses: mafernan@ubu.es (M.Á. Fernández-Muiño), mtsancho@ubu.es (M.T. Sancho).

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Abbreviations

SP	spray drying
VC	vacuum drying
FZ	freeze drying
MD	maltodextrin
AG	Arabic gum
WH	whey protein isolate
LH	Ling-heather honey
LHP	Ling-heather honey powder

2021; Sramek et al., 2016), microwave-vacuum (Cui, Sun, Chen, & Sun, 2008), vacuum puffing (Sahu & Devi, 2013), and foam-drying (Sramek et al., 2016) are also described as common drying techniques. To obtain honey powder avoiding the problems related to stickiness, the use of carriers that increase the T_g is of utmost importance. The carriers more frequently used to make honey powder are maltodextrin, Arabic gum, whey protein isolate or sodium caseinate (Samborska, Langa, Kamińska-Dwórznicka, & Witrowa-Rajchert, 2014; Suhag & Nanda, 2017). Nutriose or skimmed milk powder were also employed in order to increase the nutritional value of the honey powder (Samborska et al., 2020). Most research about dried honey aims attention at physicochemical properties such as colour, T_g, hygroscopicity, solubility, tapped density, moisture, water activity, sugar composition, proline, morphological properties and sensory features (Nurhadi et al., 2012; Osés et al., 2021; Samborska, 2019; Shi et al., 2013). There are much less studies about beneficial parameters of honey powders, being limited to total phenolics and antioxidant capacities, such as FRAP, CUPRAC, DPPH and TEAC, using different polyphenols' extraction procedures. Suhag and Nanda (2016) obtained honey powders' phenolic extract with acetone 60%, stirring 30 min at 30 °C; Bansal, Premi, Sharma, and Nanda (2017) with methanol, centrifuging 10 min; Samborska et al. (2020) with 70% acetone, shaking 2 h, and then by static extraction at 4 °C during 24 h, followed by shaking 30 min and eventually filtering. The latter procedure was modified by Jedlińska et al. (2021), dissolving the powder in water and ethanol instead using acetone 70%, while Barańska, Jedlińska, and Samborska (2021) and Tomczyk, Zaguła, Tarapatskyy, Kačániová, and Dżugan (2020) dissolved the honey powder only with distilled water. The use of different extraction procedures for honeys' phenolics makes it difficult the comparison of results among papers on dried honeys, as well as on both dried and raw honeys, in which phenolic extracts were mostly obtained employing amberlite or SPE (Baltrušaitytė, Venskutonis, & Čeksterytė, 2007; Sancho et al., 2016). All the mentioned research on phenolic extracts of dried honeys was done in powders obtained by spray drying. Only Rivero et al. (2021) assessed the antioxidant activity of honey powder obtained by lyophilization. Tomczyk et al. (2020) also evaluated the antimicrobial activity of honey powder obtained by spray drying using maltodextrin as carrier. Antioxidant activities of honey powders against free radicals particularly damaging to living organisms, such as peroxyl ($ROO^{\bullet}$), hydroxyl ($^{\bullet}OH$) and superoxide ($O_2^{\bullet-}$) have not been researched, so far. Moreover, no scientific paper has been published dealing with antioxidant, antimicrobial or anti-inflammatory activities of honey powders obtained by different drying procedures using different carries. Therefore, the aim of this study was to research to which extent the quantity of polyphenols, antioxidant, antimicrobial and anti-inflammatory activities of honeys were modified in honey powders obtained with different treatments (spray drying, vacuum drying and freeze drying) using different carriers (maltodextrin, Arabic gum and whey).

2. Materials and methods

2.1. Standards, reagents and apparatus

Arabic gum (Sigma-Aldrich, 30888). Whey protein isolate with a protein content of 90% (Myprotein, Manchester, UK). Maltodextrin of dextrose equivalent 20 (Calaf Nuances, Barcelona, Spain). Methanol, sodium carbonate, sodium chloride, sodium hydroxide, potassium hydroxide, potassium persulfate, $Fe(NH_4)_2(SO_4)_2$, EDTA, acetic acid, formic acid, *p*-dimethylaminobenzaldehyde, HCl, H_2SO_4, H_2O_2, Baird Parker agar (BP), Trypone bile x-glucuronide agar (TBX) and egg yolk tellurite sterile emulsion (VWR International Eurolab, part of Avantor, Llinars del Vallés, Spain). Gallic acid, $NaNO_2$, Na_2SO_4 and catechin (Panreac, Barcelona, Spain). $AlCl_3$ and fluorescein sodium salt (Fluka Chemie GmbH, part of Sigma-Aldrich, Buchs, San Galo, Switzerland). Na_2HPO_4, NaH_2PO_4 (Scharlab, Sentmenat, Spain). Potassium tetraborate, nitro-blue tetrazolium, sodium benzoate and uric acid (Alfa Aesar, part of Thermo Fisher, Kandel, Rheinland-Pfalz, Germany). Nutrient broth No. 2 (NB), brain heart infusion (BHI), agar technical No. 2 and Ringer solution (Oxoid, part of Thermo Fisher, Basingstoke, Hampshire, UK). Microinstant Listeria Agar Base (LAB), Listeria enrichment supplement Ottaviani & Agosti and Listeria Selective Supplement (Scharlau S.L., Senmanat, Spain). Folin-Ciocalteu phenol reagent, quercetin, dinitrophenylhydrazine, 2,2′ -azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), thiobarbituric acid, xanthine, xanthine oxidase (10110434001), 2,2′ -azobis(2-amidopropane) dihydrochloride (ABAP), *N*-acetyl-D-glucosamine (NAG), hyaluronic acid sodium salt from *Streptococcus equi* (53,747), bovine serum albumin, hyaluronidase from bovine testes type IV-S (1400 U/ml, H3884) (Sigma-Aldrich, part of Merck, Steinheim, Nordrhein-Westfalen, Germany). Water was deionized using a Milli-Q water purification system (Millipore, part of Merck, Bedford, MA, USA). Absorbances were measured with a 400Bio UV–visible spectrophotometer (Varian, Mulgrave, Vic., Australia). Fluorescence measurements were measured with a Varioskan LUX microplate reader (Thermo Fisher).

2.2. Samples

Sampling comprised three ling-heather (*Calluna vulgaris* (L.) Hull) honeys (LH), named as LH1, LH2 and LH3 and their corresponding powders (LHP) named as LHP1, LHP2 and LHP3. Honeys' botanical origins were ascertained by both melissopalinology (Louveaux, Maurizio, & Vorwohl, 1978; Terradillos, Muniategui, Sancho, Huidobro, & Simal-Lozano, 1994; Von der Ohe, Persano Oddo, Piana, Morlot, & Martin, 2004) and sensory analyses (Marcazzan, Mucignat-Caretta, Marchese, & Piana, 2018; Persano Oddo & Piro, 2004; Piana et al., 2004). LH1 and LH2 were heather honeys, in which the relative frequency of the sum of bell-heather (*Erica* spp.) and ling-heather pollen was higher than 45%, being the relative frequency of ling-heater pollen higher than 20%. In contrast, the relative frequency of the sum of bell-heather and ling heater pollens in LH3 did not reach 45%, being the relative frequency of ling-heater pollen higher than 15%. *Calluna vulgaris* (L.) Hull pollen can be under-represented in ling-heather honeys (Von der Ohe et al., 2004), so that samples with 10% relative frequency *Calluna* pollen can be ling-heather unifloral honeys if their attributes correspond with those of ling-heather. The three samples (LH1, LH2 and LH3) had noticeable sensory properties of ling-heather honeys, such as dark reddish tone, woody warm floral flavour, medium bitternes and long aftertaste persistence (Persano Oddo & Piro, 2004), among others, so that all of them could be commercialized as unifloral ling-heather honeys. Powders (27 samples) were obtained with three drying methods: spray drying (SP), vacuum drying (VC) and freeze drying (FZ), using for each procedure three different carriers: Arabic gum (AG), whey protein isolate (WH) and maltodextrin (MD). All drying procedures were described in detail in a previous paper (Osés et al., 2021), as well as in

the supplementary file (S1).

2.3. Analysis of biological properties

In LH and LHP, total phenolics, flavonoids, antioxidant activity against four radicals, anti-inflammatory and antimicrobial activity against *Staphylococcus aureus*, *Listeria monocytogenes* and *Escherichia coli* were determined in triplicate.

2.3.1. Phenolic extracts

Phenolic compounds of LH (5.00 g) and LHP (2.00 g) samples dissolved in 20 mL acidified distilled water (pH = 2, with HCl), were extracted by solid phase extraction (SPE) with 5 mL methanol (Sancho et al., 2016), using SPE Strata-X PRO columns (200 mg/6 ml; Phenomenex, Torrance, CA, USA) and a manifold (Phenomenex). The extracts were kept frozen (−30 °C) in amber glass bottles until analysis.

2.3.2. Total phenolics' content (TPC)

TPC were determined in LH, LHP and their corresponding methanolic extracts (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Honey (10.0 g/100 ml distilled water) and powders (0.5 g in 5 ml of distilled water) samples were filtered through a Whatman nº 40. The samples (100 μL of dissolved LH and LHP or 100 μL of methanolic extracts), were mixed with 500 μL 0.2 mol/L Folin-Ciocalteu reagent. After 5 min, 400 μL of $NaCO_3$ (75 g/L) were added and the samples were incubated 2 h at room temperature. Absorbance was measured at 760 nm, using gallic acid (GA) as standard for the calibration curve (5–500 mg/L). Results were expressed as mg GA/100 g dry honey or 100 g dry honey powder.

2.3.3. Total flavonoids

LH and LHP flavonoids' determinations were carried out on methanolic extracts. Two types of flavonoids were determined flavone/flavonol and flavanols.

Flavone/Flavonol content was analysed by the reaction of these flavonoids with $AlCl_3$ in neutral medium (Meda et al., 2005). 500 μL methanolic extracts were mixed with 500 μL $AlCl_3$ (2% in methanol). After 10 min the absorbance was read at 415 nm, using quercetin (Q) as standard for the calibration curve (1–200 μg/ml). For each sample colour correction was needed, replacing $AlCl_3$ by methanol. Results were expressed as mg Q/100 g dry honey or 100 g dry honey powder.

Flavanols were analysed by reacting with $AlCl_3$ in alkaline medium (Pękal & Pyrzynska, 2014). 500 μL methanolic extracts were mixed with 150 μL $NaNO_2$ (5 g/100 mL). After 5 min, 250 μL $AlCl_3$ (2 g/100 mL in methanol) was added and mixed. After 6 min, 250 μL NaOH 1 mol/L was added. After 10 min at room temperature, the absorbance was read at 510 nm, using catechin (Ct) as standard for the calibration curve (1–100 μg/ml). Results were expressed as mg Ct/100 g dry honey or 100 g dry honey powder.

2.3.4. Antioxidant activities

Antioxidant capacity by $ABTS^{\bullet+}$ scavenging activity test: Trolox equivalent antioxidant capacity (TEAC) of LH (0.5 g in 1 ml of water) and LHP (0.1 g in 1 ml of water) was evaluated following the steps detailed in the paper of Osés et al. (2020) based on the method of Re et al. (1999) modified by Sancho et al. (2016). $ABTS^{\bullet+}$ scavenging by the sample was spectrophotometrically measured at 734 nm, using Trolox (T) as standard for the calibration curve (0.625–3 mmol/L). Results were expressed as μmol T/100 g dry honey or 100 g of dry honey powder.

The concentration of aqueous LH and LHP solutions was 0.1 g/ml for the measurement of the oxygen radical absorbance capacity (ORAC), which determines antioxidant activity against $ROO^{\bullet}$. Antioxidant capacities as radical-scavenging effect on $^{\bullet}OH$ (AOA) and $O_2^{\bullet-}$ (SRS) were assessed on 75% LH and 75% LHP aqueous solutions. ORAC, AOA and SRS analytical procedures were carried out following the steps detailed by Osés et al. (2020).

ORAC was determined by fluorescence (being excitation and emission wavelengths 485 nm and 520 nm, respectively). Peroxyl radicals reacted with fluoresceine disodium releasing a nonfluorescent product. In a system, in which the diluted samples LH and LHP had been added, fluorescence was measured following Osés et al. (2020) procedure. Either to 187 μl sample diluted in buffer (75 mmol/L sodium phosphate, pH 7.4), or to 187 μl buffer (75 mmol/L sodium phosphate, pH 7.4) or to a mixture of 181 μl buffer (75 mmol/L sodium phosphate, pH 7.4) and 6 μl 0.2 μmol/L T solutions, 3 μl 4.1 μmol/L fluorescein disodium was added, in a 96-well white plate (Greiner Bio-one, San Sebastian de los Reyes, Madrid, Spain) keeping the plate at 37 °C, 5 min. Then, 10 μl, 0.37 mol/L ABAP was added to the mixture and measured at 37 °C every 5 min throughout 90 min. The results were expressed as μmol T/g dry honey or g of dry honey powder using the following equation:

ORAC (μmol T/g) = [(area sample – area blank)/((area T - area blank)/μmol T)]

AOA was carried out by Koracevic, Koracevic, Djordjevic, Andrejevic, and Cosic (2001) method, which spectrophotometrically (532 nm) measured the ability of the diluted samples (75% LH and 75% LHP) for overriding the TBARS (thiobarbituric acid reactive substances), released from the reaction of $^{\bullet}OH$ and benzoate. $^{\bullet}OH$ were produced by a Fenton type reaction of H_2O_2 plus Fe-EDTA complex. 1 mmol/L uric acid (UA) in NaOH (5 mmol/L) was used as standard, expressing the results as mmol UA/100 g dry honey or 100 g dry powder.

SRS: $O_2^{\bullet-}$ was generated by the xanthine-xanthine oxidase system (Küçük et al., 2007). The capacity of the diluted samples LH and LHP for inhibiting $O_2^{\bullet-}$ was spectrophotometrically measured (560) nm, expressing the results as % inhibition = (A – (M-B)/A) x 100, where A was the absorbance in the positive control (in which buffer replaced sample), M was the absorbance of each sample reaction and B was the absorbance of blank samples (in which buffer replaced the enzyme).

2.3.5. Anti-inflammatory activity

Anti-inflammatory activity of LH and LHP (75% in water) was assessed by hyaluronidase inhibition assay (Ferreres et al., 2012), based on the mechanism of the Morgan-Elson reaction with few modifications described by Osés et al. (2020). Hyaluronidase activity was defined as 1 unit (U) of hyaluronidase that catalyzes the liberation of 1 μmol NAG per min under specified conditions. NAG standard solutions (in the range between 0 and 2 μmol per test), were used as standard for calibration curves. Absorbance was read at 586 nm against a blank, in which buffer replaced hyaluronidase. With the NAG produced by each enzymatic reaction and using the linear regression equation, the percentage of enzyme inhibition was calculated as % Inhibition = (A - B/A) x 100, where A was μmol NAG in the positive control (in which buffer replaced sample), and B was μmol NAG of each sample reaction.

2.3.6. Antimicrobial activity

Antimicrobial activity of LH and LHP were assayed by broth microdilution method against three bacteria: *Staphylococcus aureus* CECT 435, *Escherichia coli* CECT 99 and *Listeria monocytogenes* CECT 934 (Spanish Type Culture Collection, Valencia University, Spain). Stock cultures were maintained on NB for *St. aureus* and *E. coli* or BHI for *L. monocytogenes*, with glycerol (20%) at −80 °C. Bacterial inoculum was prepared in NB or BHI at 37 °C, 24 h. Sterile Ringer was used to prepare the cell suspensions to 6 log CFU/ml (determined using plate counts). Sterile 96 well round bottomed polystyrene microtitre plates (Brand, Wertheim, Germany) were used.

For each sample, 8 different LH and LHP concentrations (70 g/100 mL, 60 g/100 mL, 50 g/100 mL, 40 g/100 mL, 30 g/100 mL, 20 g/100 mL, 10 g/100 mL and 5 g/100 mL) were studied. Each sample at each concentration was dissolved in the corresponding broth for each bacterium. Then, 20 μl of the corresponding microorganism (6 log CFU/ml) was inoculated into a final volume of 200 μl, obtaining a final 5 log CFU/

ml microorganism concentration (verified using plate counts). The 96 bottomed plates were incubated at 37 °C, 24 h. After incubation, a turbidity due to the precipitate produced by the microorganism growth was observed. The minimal inhibitory concentration (MIC) was described as the minimal LH or LHP concentration at which no bacterium growth was visible on the plate. Eventually, 10 μl from each bottomed plate was incubated at 37 °C, 24 h, in plates with specific agar for each microorganism (BP for *St. aureus*, LAB for *L. monocytogenes* and TBX for *E. coli*), thus determining the minimal bactericidal concentration (MBC), described as the minimal LH or LHP concentration at which no bacterium was visible on the plate.

2.4. Statistical analysis

One-way analysis of variance (ANOVA) and multifactor ANOVA were use followed by Tukey's honestly significant difference test ($p < 0.05$). Pearson correlations, principal components analysis (PCA) and cluster analysis (furthest neighbour method and squared Euclidean distance), were applied to the results. Statistical software Statgraphics Ceturion XVIII (Statgraphics Technologies, Inc., The Plains, VA, USA) was used.

3. Results and discussions

All data, except % inhibition (SRS and anti-inflammatory activity) and MBC were expressed on dry basis (d.b.) for a proper comparison of LH and LHP data.

3.1. Phenolics, flavonoids and biological properties of ling heather honeys

Table 1 shows averages and standard deviations of the results corresponding to LH. TPC varied between 81 and 272 mg GA/100 g d.b. TPC of methanolic extracts were quite lower (11–22.3 mg GA/100 g d.b.). These values agreed with the results obtained by other authors that ranged between 110 and 195 mg GA/100 g in heather honeys and between 15.6 and 60.3 mg GA/100 g in extracts (Kaygusuz et al., 2016; Sancho et al., 2016; Starowicz, Ostaszyk, & Zieliński, 2021). Lower TPC of methanolic extracts were expected, because Folin-Ciocalteu reagent also quantifies other reducing compounds, such as sugars (Petretto, Cossu, & Alamanni, 2015) that are present in crude samples, but not in their methanolic extracts.

Flavones and flavonols ranged between 0.71 and 1.69 mg Q/100 g d.b., which were lower than the ranges obtained by other researchers in heather honeys with values between 2.4 and 21 mg Q/100 g (Aazza, Lyoussi, Antunes, & Miguel, 2013; Kivima et al., 2021; Rodríguez-Flores, Escuredo, Seijo-Rodríguez, & Seijo, 2019). Flavanols ranged between 2.08 and 4.42 mg Ct/100 g d.b.

TEAC, ORAC, AOA and SRS exhibited by LH samples brought to light that TEAC (20–825 μmol T/100 g d.b.), and AOA (<0.15 mmol UA/100 g d.b.) results were similar to those obtained by other researchers (Gorjanović et al., 2013; Osés, Pascual-Maté, Fernández-Muiño, López-Díaz, & Sancho, 2016) in different honeys. However, higher ORAC values than those obtained in this study were described in other papers, with results of 22.6 μmol T/g d.b. for heather honeys (Aazza et al., 2013). Regarding SRS, LH (750 mg/ml) showed a $O_2^{\bullet-}$ inhibition between 25% and 78%. In another study carried out on chestnut, rhododendron and heterofloral honeys, the IC_{50} ranged between 5.2 and 7.6 mg/ml (Küçük et al., 2007).

Anti-inflammatory activity of LH ranged from 46.6 to 54.2%, being around 0.75 g/ml (75%) the IC_{50}. The results for this activity were similar to the anti-inflammatory capacities described by Osés et al. (2016) and Osés et al. (2020) for other Spanish Ericaceae honeys, but lower than the data of Kolayli, Sahin, Can, Yildiz, and Sahin (2016) obtained in Turkish heather honeys (0.17–0.22 g/ml).

The three LH showed antimicrobial activity against the three studied pathogens. MBC varied between <5% and 40%. *St. aureus* was the most sensitive microorganism, and *E. coli* the most resistant one. For heather honeys, other researchers obtained MBC ranging from 4.7 to 37.5% against *St. aureus* (Osés et al., 2016; Salonen, Virjamo, Tammela, Fauch, & Julkunen-Tiitto, 2017).

Table 1
Averages and standard deviations (n = 3) of total phenols, flavonoids, antioxidant activities against $ABTS^{\bullet+}$, $ROO^{\bullet}$, $O_2^{\bullet-}$ and $^{\bullet}OH$ radicals, anti-inflammatory and antimicrobial activities against *St. aureus*, *L. monocytogenes* and *E. coli* of ling-heather honeys (LH1, LH2, and LH3) used to make the honey powders. All results are expressed on dry basis.

	LH1	LH2	LH3
Total phenols (mg GA/100g)	272 ± 15^{a}	169±1^{b}	81±3^{c}
Total phenols in methanolic extract (mg GA/100g)	22.3 ± 0.4^{a}	21.4 ± 0.1^{b}	11.0 ± 0.4^{c}
Total flavones/flavonols (mg Q/100g)	1.50 ± 0.03^{b}	1.69 ± 0.05^{a}	0.71 ± 0.01^{c}
Total flavanols (mg Ct/100 g)	4.24 ± 0.19^{a}	4.42 ± 0.05^{a}	2.08 ± 0.06^{b}
TEAC ($ABTS^{\bullet+}$) (μmol T/100g)	240 ± 23^{b}	449 ± 21^{a}	123±2^{c}
ORAC ($ROO^{\bullet}$) (μmol T/g)	37.0 ± 4.6^{b}	44.0 ± 1.1^{a}	36.3 ± 1.4^{b}
AOA ($^{\bullet}OH$) (mmol UA/100 g)	0.047 ± 0.010^{a}	0.049 ± 0.008^{a}	0.052 ± 0.012^{a}
SRS ($O_2^{\bullet-}$) (% inh)	53±1^{b}	78±1^{a}	25±2^{c}
Anti-inflammatory activity (% inh)	54.2 ± 0.3^{a}	46.6 ± 0.7^{c}	53.0 ± 0.3^{b}
MBC *St. aureus*	40±0^{a}	5±0^{b}	<5±0^{b}
MBC *L. monocytogenes*	20±0^{a}	10±0^{b}	20±0^{a}
MBC *E. coli*	40±0^{a}	20±0^{b}	20±0^{b}

inh = inhibition; Different lowercase letters (a-c) indicate significant differences ($p < 0.05$) between honey.

3.2. Powdered honeys

3.2.1. Phenolics and flavonoids

Both WH and WH-LHP clearly showed the highest TCP (Fig. 1A). WH increased the values obtained for the corresponding LH between 300 and 1300%. The very high values of TPC obtained for WH-LHP were probably due to the high proteins' content of WH (>90%) that also reacted with Folin-Ciocalteu reagent (Singleton, Orthofer, & Lamuela-Raventós, 1999). AG-LHP and MD-LHP exhibited retentions between 39 and 106% compared to raw LH honeys. In comparison with MD-LHP and WH-LHP, AG-LHP showed higher TPC by SP and VC. Regarding FZ, no significant differences were found between TPC of FZ-AG-LHP and FZ-MD-LHP, probably because of the amount of honey in the powdered samples that was around 75% honey in all FZ-LHP. In contrast, the content of honey in SP-AG-LHP was around 60%, in VC-AG-LHP was around 75%, in SP-MD-LHP was around 50% and in VC-MD-LHP was around 60%. With regard to the dehydration technique, in general VC-LHP exhibited the highest TPC, probably due to the Maillard reaction compounds formed during the dehydration procedure at 60 °C/72 h. Therefore, drying procedure, carrier used and their interaction significantly influenced TPC (Table 2).

In order to eliminate the interference of sugars (from honey), and proteins (mainly from WH), in analysis of TPC, measurements of this parameter were carried out in methanolic extracts (Fig. 1B). As expected, TPC results were lower in powders' extracts than in LHP. In contrast to drying procedures, studied carriers and their interaction with drying procedures showed no influence on TPC (Table 2). LHP extracts obtained from LH3 exhibited the lowest values (5–16 mg GA/100 g d.b.), while the extracts obtained from LH1 showed the highest results (22–37 mg GA/100 g d.b.), which were in accordance with the data of LH. Similar values (28.6 mg GA/100 g) were obtained by Samborska et al. (2020) for SP honey powders obtained with MD at 50%, while Suhag and Nanda (2016) and Tomczyk et al. (2020) obtained higher TPC (29.6–51.1 mg GA/100 g) in SP powders with AG, WH or MD. These slight differences could be due to the use of different honeys, but also to different phenol's extraction procedures used by each research team. Higher TPC were obtained by other authors (Suhag et al., 2016), adding

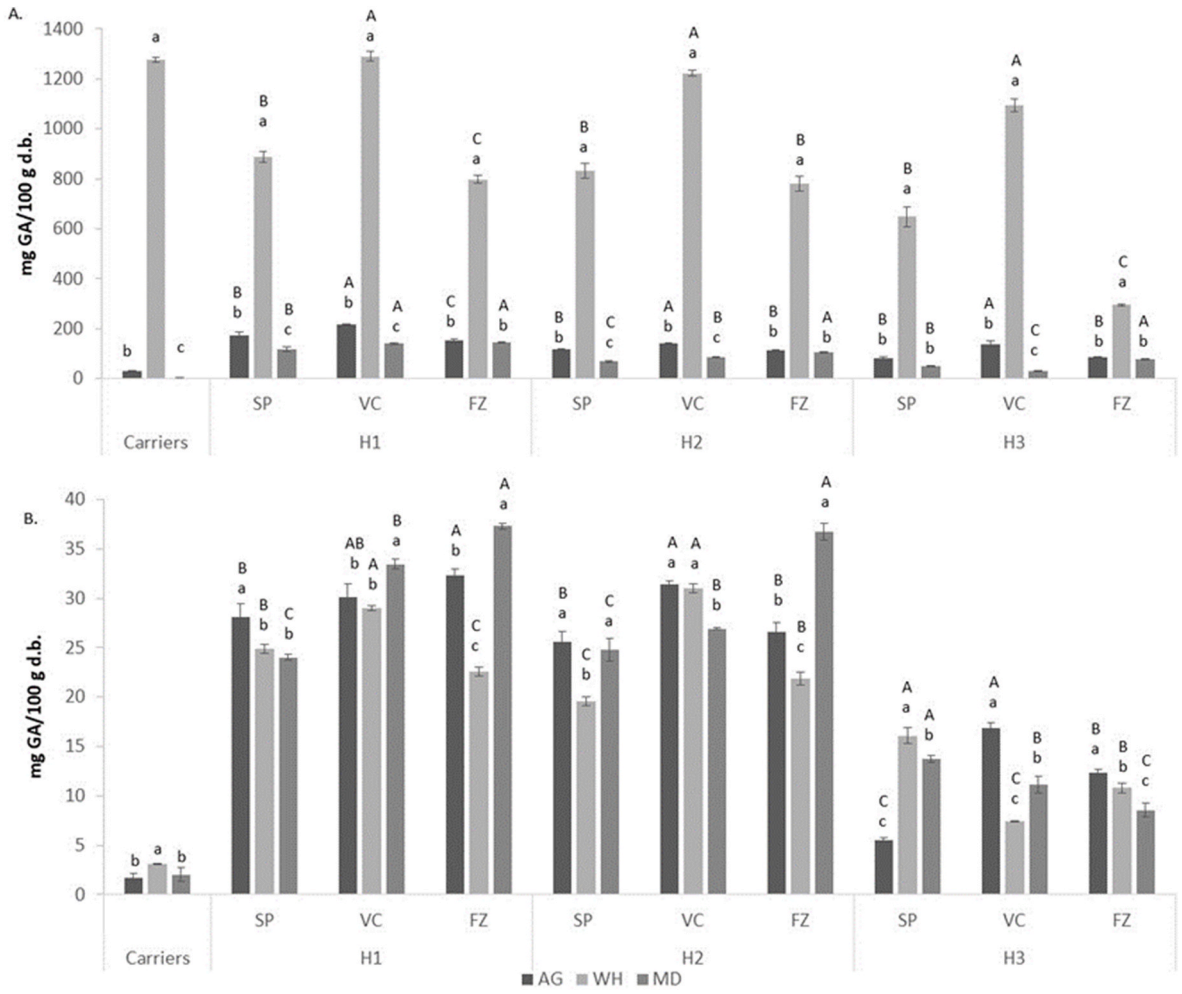

Fig. 1. Total phenols (mg of GA/100 g dry basis (d.b.)) of carriers and honey powders (A) and their methanolic extracts (B) obtained by spray (SP), vacuum (VC) and freeze (FZ) drying, using Arabic gum (AG), whey (WH) and maltodextrin (MD) (n = 3). Error bars represent the standard deviation for each data point. Different capitals letters (A–C) indicate significant differences ($p < 0.05$) between drying procedures for each honey and carrier. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) between carriers for each honey and drying procedure.

Table 2
Effect of interaction (Multifactor ANOVA), between drying procedures and carriers for phenols, flavonoids, antioxidant, anti-inflammatory and antimicrobial activities.

Source	TPC	TPCex	Flavon.	Flavan.	$ABTS^{\bullet+}$	$ROO^{\bullet}$	$^{\bullet}OH$	$O_2^{\bullet-}$	Anti-infl.	*St. aureus*	*L. mono*	*E. coli*
A: Drying	***	*	NS	*	***	*	***	***	NS	NS	NS	*
B: Carrier	***	NS	NS	*	***	***	***	***	***	**	NS	NS
Interaction: AB	***	NS	NS	NS	***	NS	***	*	*	NS	***	*

TPC: Total phenolics' content; TPCex: Total phenolics' content in methanolic extracts; Flavon.: Flavone/Flavonol; Flavan: Flavanols. Anti-infl.: anti-inflammatory; L. mono: *L. monocytogenes.*
* p-value < 0.05.
** p-value < 0.01.
*** p-value < 0.001.
NS: non significant.

aonla and basil extracts to the honey powders (60–62 mg GA/100 g); or using skimmed milk powder (Samborska et al., 2020) as carrier (50–70 mg GA/100 g). Powders' TPC showed a retention between 50 and 172% in comparison to raw honeys, and only three LHP were below 90% retention. The results of LHP were higher than those obtained by Jedlińska et al. (2021) in SP honey powders using Nutriose as carrier in a ratio 1:1 (retention of 41–59% in SP powders, and 80–91% using dehumidified air SP), probably because of the use of different honey concentration, different carriers and different method for phenols extraction. Therefore, regardless the drying method, all the LHP retained the TPC and none of the used drying procedure deteriorated honey phenolics compounds. Conversely, other researchers observed lower TPC in honeys dehydrated by SP at high temperature (Suhag et al., 2016).

Total flavanols in LHP ranged from 0.38 to 36.3 mg Ct/100 g d.b. Total flavone/flavonols ranged between 0 and 3.6 mg Q/100 g d.b. (Fig. 2). These results were higher than those obtained for raw honeys. Although all carriers seemed to somehow contribute or influence the flavonoids' analyses, with regard to flavanols, results were neither related to the drying method nor to the type of carrier. With regard to

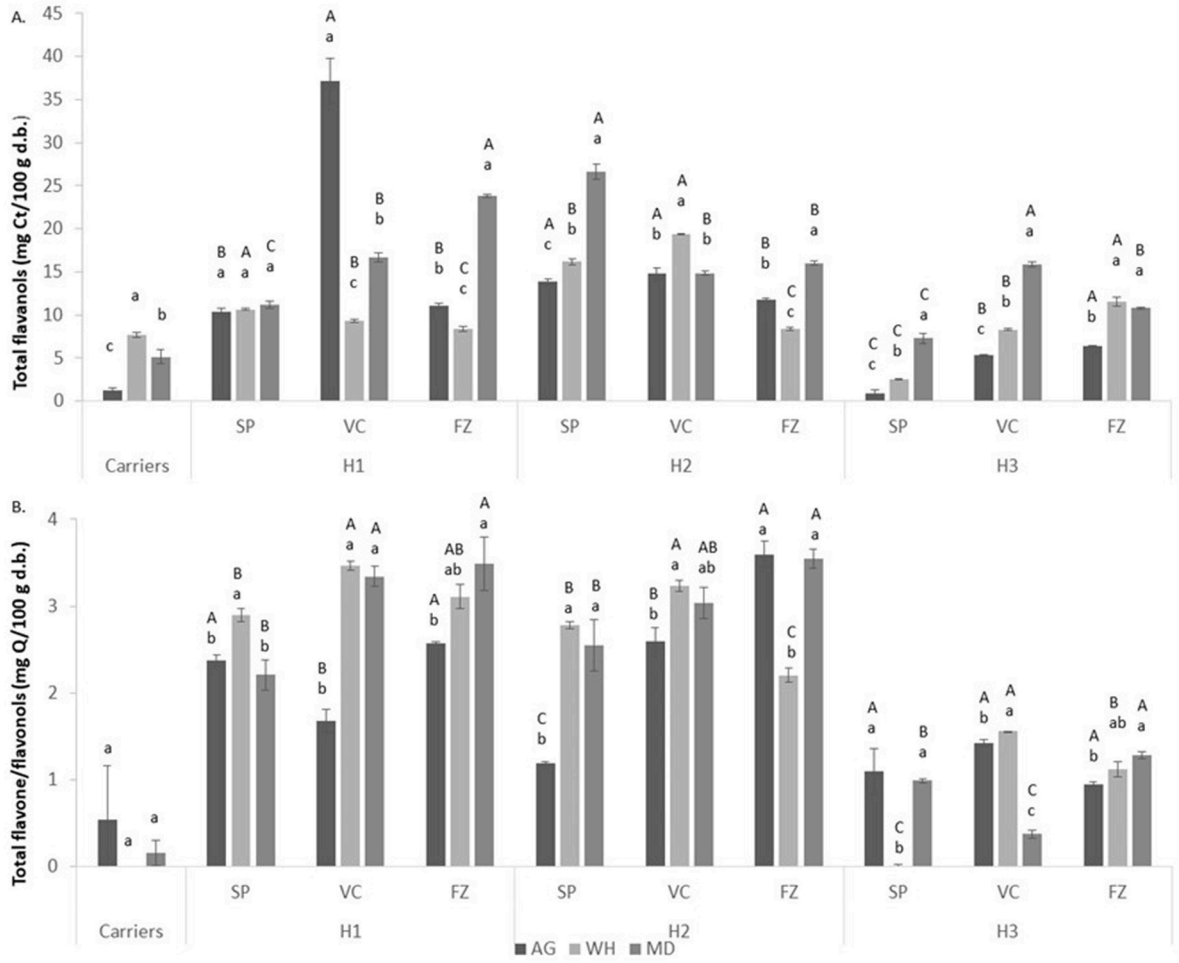

Fig. 2. Total flavanols (mg of Ct/100 g dry basis (d.b.)) and total flavone/flavonols (mg of Q/100 g dry basis (d.b.)) of carriers and honey powders obtained by spray (SP), vacuum (VC) and freeze (FZ) drying, using Arabic gum (AG), whey (WH) and maltodextrin (MD) (n = 3). Error bars represent the standard deviation for each data point. Different capitals letters (A–C) indicate significant differences ($p < 0.05$) between drying procedures for each honey and carrier. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) between carriers for each honey and drying procedure.

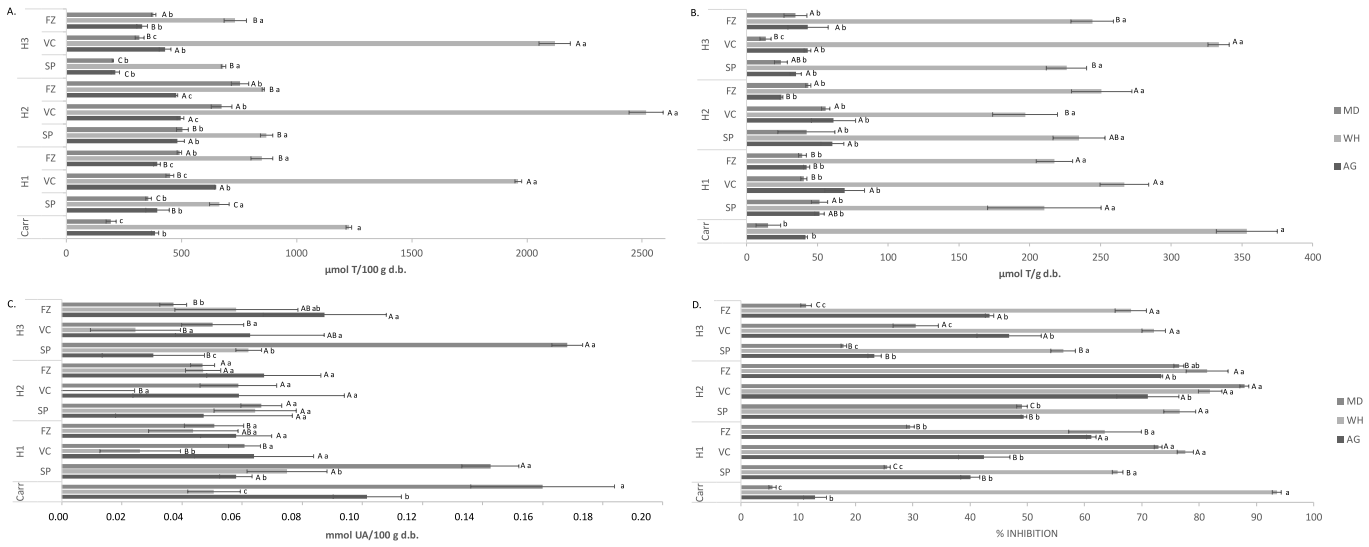

Fig. 3. Antioxidant activity of carriers (Carr) and honey powders obtained by spray (SP), vacuum (VC) and freeze (FZ) drying, using Arabic gum (AG), whey (WH) and maltodextrin (MD) against different radicals ($ABTS^{\bullet+}$ (A), $ROO^{\bullet}$ (B), $^{\bullet}OH$ (C) and $O_2^{\bullet-}$ (D)). Error bars represent the standard deviation for each data point. Different capitals letters (A–C) indicate significant differences ($p < 0.05$) between drying procedures for each honey and carrier. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) between carriers for each honey and drying procedure (n = 3).

flavones, results were slightly influenced by both factors, but not by their interaction (Table 2). The obtained results confirmed that the total amount of different flavonoids of honeys were completely retained in the corresponding honey powders.

3.2.2. Antioxidant activity

Fig. 3 shows the antioxidant activity of all carriers and LHP against the four studied radicals. Antioxidant activity of honey powders has not been extensively studied, so far. Only four researchers determined TEAC capacity against $ABTS^{\bullet+}$ (Barańska et al., 2021; Mutlu & Erbas, 2021; Rivero et al., 2021; Samborska et al., 2020), not there being any study against peroxyl, hydroxyl and superoxide radicals. In this study, TEAC (Fig. 3A) ranged between 201 and 754 μmol T/100 g d.b. for AG-LHP and MD-LHP, and between 646 and 2518 μmol T/100 g d.b. for WH-LHP. TEAC of LHP were higher than those of LH. LHP3 showed the lowest TEAC, likewise its corresponding honey (LH3). All the carriers exhibited high TEAC results, being partly responsible for the LHP anti-$ABTS^{\bullet+}$ capacities (Table 2). WP-LHP exhibited the highest TEAC values, which could be attributed to the antioxidant activity of WH, due to its hydrophobic and aromatic amino acids that can stabilize electron deficient radicals by donating protons (Arranz et al., 2019). Similar results were reported by Mutlu and Erbas (2021) in honey-like powder obtained by VC and SP, with TEAC values of 251 μmol T/100 g in powders containing MD, 787 μmol T/100 g in powders containing AG and 3017 μmol T/100 g in powders containing WH. Our results were also comparable to those of Samborska et al. (2020), with a TEAC of 726 mg T/kg (= 290 μmol T/100 g solids) in a honey powder obtained by SP using MD, and values from 1080 to 1245 mg T/kg (= 431–497 μmol T/100 g solids) in honey powders using skimmed milk as carrier. In honey powders obtained by FZ using different WH and AG ratios, Rivero et al. (2021) described TEAC values ranging from 5 to 10 mmol T/100 g, also observing higher antioxidant activities in honey powders than in the corresponding raw honeys (0.7–2.3 mmol T/100 g). These researchers concluded that TEAC was mostly related to the type of honey and its origin, not being much affected by the carrier ratio. In general, with regard to the dehydration technique, VC-LHP provided the highest TEAC, probably due to Maillard compounds that could contribute to increase antioxidant activities against $ABTS^{\bullet+}$ (Suhag & Nanda, 2017). Mutlu and Erbas (2021) also described higher TEAC values for honeys dehydrated by vacuum drying, in comparison with honey powders obtained by other methods. TEAC results showed to be highly influenced by the interaction between carriers and drying procedures (Table 2).

ORAC ranged between 197 and 333 μmol T/g d.b. for WH-LHP, from 24.5 to 69.2 μmol T/g d.b for AG-LHP and from 13.4 to 55.9 μmol T/g d.b for MD-LHP, obtaining the following ORAC in decreasing order WH > AG > MD (Fig. 3B). This order could be related to the carrier composition, as well as to the honey concentration, because SP-WH-LHP and VC-WH-LHP samples had higher honey concentrations (75%) than SP-MD-LHP (50% honey) and VC-MD-LHP (60% honey). An ORAC value of 23000 μmol T/100 g was reported for WH (Adjonu, Doran, Torley, & Agboola, 2013).

AOA showed an enormous variability between samples and even for the same sample (Fig. 3C). AOA results varied between 0 and 0.168 mmol UA/100 g d.b., being in almost all LHP higher than in LH (Table 1). AOA seemed to be very influenced by the carrier (Table 2), because MD exhibited the highest values, followed by AG, and then, by WH. Conversely to what occurred with other antioxidant capacities, WH did not show the highest AOA results, highlighting the importance of using several methods against different free radicals to determine the antioxidant activity, because no single method is capable of properly assessing all antioxidant capacities. In general, with regard to the dehydration technique, SP-LHP showed the highest AOA values, meaning that the drying technique also influenced this activity, likewise the interaction between carriers and drying procedures (Table 2).

LHP showed an SRS between 11% and 88%, presenting LHP2 the highest radical inhibition (Fig. 3D), agreeing with the result of LH2 (Table 1). WH exhibited an inhibition of 94%, obtaining WH-LHP the highest inhibition percentage. Regarding the drying technique, SP-LHP showed the lowest inhibition, and VC-LHP the highest. Other authors assessed antioxidant activity of honey powders against other free radicals, such as DPPH or FRAP (Jedlińska et al., 2021; Suhag et al., 2021; Suhag & Nanda, 2016; Tomczyk et al., 2020), concluding that when SP was used, the more the inlet temperature increased, the lower the antioxidant activity was, because at high temperatures the phenols, which are mainly responsible for antioxidant capacity, were easily oxidized and degraded. These researchers also claimed that WH promoted an increase of the antioxidant activity due to its superior encapsulating properties.

The lower values of total phenols, flavonoids, TEAC and SRS obtained for sample LH3 and its corresponding powders could be due to the less purity in heather of LH3 in comparison with LH1 and LH2, even although all samples were ling-heather unifloral honeys.

3.2.3. Anti-inflammatory activity

Fig. 4 shows the anti-inflammatory activity of the carriers and LHP. Although, carriers did not exhibit any anti-inflammatory activity by hyaluronidase inhibition assay, the use of different carriers seemed to affect the retention of this honey activity, not there being a relation with the drying procedure, or with the percentage of honey in the powder. AG-LHP showed the highest inhibition percentage, meaning that there was a complete retention of the raw honeys anti-inflammatory activities (91–137%). However, in comparison with the corresponding LH anti-inflammatory activities, MD-LHP retained between 77 and 145% anti-inflammatory activity, and WH-LHP only between 6 and 17% anti-inflammatory activity. Therefore, WH showed the lowest retention of the components responsible for this activity. In strawberry tree honeys, Osés et al. (2020) reported that honey anti-inflammatory activity was mainly related to non-phenolic compounds, such as some volatile and semivolatile substances.

3.2.4. Antimicrobial activity

The antimicrobial activity was evaluated in carriers and LHP at concentrations between 5 and 70% using the broth microdilution method (Table 3). Carriers did not show antimicrobial activity at that concentrations, while LHP showed MBC between 10 and > 70%, which were lower antimicrobial activities than those of their corresponding LH. *E. coli* was the microorganism most sensitive to LHP, while *L. monocytogenes* was the most resistant. Likewise, to what occurred with LH, LHP1 showed the lowest antimicrobial activities, being these capacities similar in LHP2 and LHP3. WH-LHP and MD-LHP showed higher activities than AG-LHP against *St. aureus* (Tables 2 and 3). With regard to the drying procedures, FZ-LHP showed higher antimicrobial activities than SP-LHP and VC-LHP against *E. coli* (Tables 2 and 3), which could be due to the fact that during VC and SP samples are heated, in contrast to FZ, which takes place in cold. Interaction between carriers and drying procedures influenced antimicrobial activities against *L. monocytogenes* and *E. coli* (Table 2). Tomczyk et al. (2020) also assayed the antimicrobial activity by agar well diffusion of honey powders obtained by SP using MD at 50%. These researchers observed that none of the samples showed any antibacterial action against the microorganisms tested, which could be due to losses of honey antioxidant thermolabile components, to the 50% MD in the final product, or to the powder concentrations tested (12.5–50%) that were not enough to provide antibacterial activities.

4. Statistical analysis

At 95% significance, TPCs were linearly correlated to TEAC (r = 0.8474), ORAC (r = 0.9136) and SRS (r = 0.617), whereas methanolic extracts' TPCs were linearly correlated to flavanols (r = 0.4823) and flavone/flavonols (r = 0.7190). These results bring to light that antioxidant activities are related to phenolic compounds, as well as to other

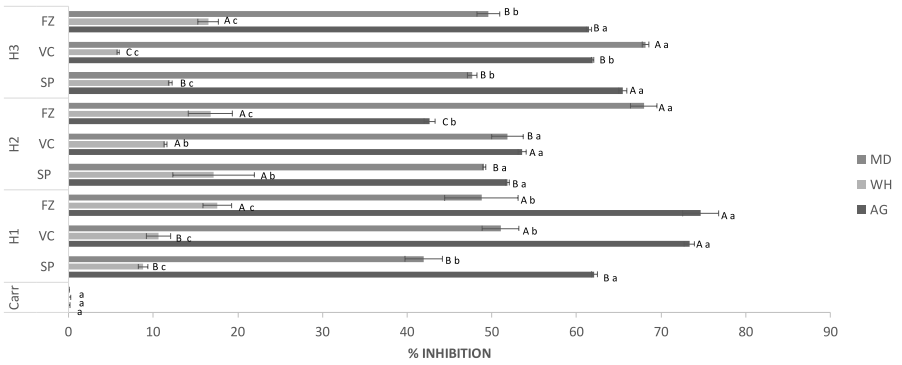

Fig. 4. Anti-inflammatory activity of carriers and powdered honeys obtained by spray (SP), vacuum (VC) and freeze (FZ) drying, using Arabic gum (AG), whey (WH) and maltodextrin (MD). Error bars represent the standard deviation for each data point. Different capitals letters (A–C) indicate significant differences ($p < 0.05$) between drying procedures for each honey and carrier. Different lowercase letters (a–c) indicate significant differences ($p < 0.05$) between carriers for each honey and drying procedure ($n = 3$).

Table 3
Minimal bactericidal concentration (MBC) of carriers and honey powders obtained by spray (SP), vacuum (VC) and freeze (FZ) drying, using Arabic gum (AG), whey (WH) and maltodextrin (MD) expressed as % honey against *St. aureus*, *L. monocytogenes* and *E. coli*. Triplicates show identical MBC for each sample. Different MBC values indicate significant differences ($p < 0.05$) among samples.

St. aureus										
		LHP1			LHP2			LHP3		
		SP	VC	FZ	SP	VC	FZ	SP	VC	FZ
AG	>70	$^{A}70\pm0_{b}$	$^{A}70\pm0_{b}$	$^{B}60\pm0_{b}$	$^{A}50\pm0_{a}$	$^{A}50\pm0_{a}$	$^{A}50\pm0_{a}$	$^{A}70\pm0_{a}$	$^{B}60\pm0_{a}$	$^{A}70\pm0_{a}$
WH	>70	$^{A}>70_{a}$	$^{B}70\pm0_{b}$	$^{A}>70_{a}$	$^{B}20\pm0_{c}$	$^{A}50\pm0_{a}$	$^{B}20\pm0_{b}$	$^{B}20\pm0_{b}$	$^{A}30\pm0_{c}$	$^{B}20\pm0_{b}$
MD	>70	$^{A}>70_{a}$	$^{A}>70_{a}$	$^{A}60\pm0_{b}$	$^{A}30\pm0_{b}$	$^{A}30\pm0_{b}$	$^{B}20\pm0_{b}$	$^{B}20\pm0_{b}$	$^{A}50\pm0_{b}$	$^{C}10\pm0_{c}$
L. monocytogenes										
		LHP1			LHP2			LHP3		
		SP	**VC**	**FZ**	**SP**	**VC**	**FZ**	**SP**	**VC**	**FZ**
AG	>70	$^{B}70\pm0_{b}$	$^{B}67\pm6_{b}$	$^{A}>70_{a}$	$^{B}37\pm6_{b}$	$^{B}37\pm6_{c}$	$^{A}>70_{a}$	$^{A}70\pm0_{a}$	$^{B}50\pm0_{b}$	$^{B}47\pm6_{b}$
WH	>70	$^{A}>70_{a}$	$^{A}>70_{a}$	$^{A}>70_{a}$	$^{C}20\pm0_{c}$	$^{B}47\pm6_{b}$	$^{A}>70_{a}$	$^{C}20\pm0_{c}$	$^{A}>70_{a}$	$^{B}57\pm6_{a}$
MD	>70	$^{A}70\pm0_{b}$	$^{A}70\pm0_{b}$	$^{B}60\pm0_{b}$	$^{A}70\pm0_{a}$	$^{A}70\pm0_{a}$	$^{B}57\pm6_{b}$	$^{B}60\pm0_{b}$	$^{A}>70_{a}$	$^{C}20\pm0_{c}$
E. coli										
		LHP1			LHP2			LHP3		
		SP	**VC**	**FZ**	**SP**	**VC**	**FZ**	**SP**	**VC**	**FZ**
AG	>70	$^{A}50\pm0_{b}$	$^{B}40\pm0_{b}$	$^{B}40\pm0_{b}$	$^{A}40\pm0_{a}$	$^{A}40\pm0_{a}$	$^{B}30\pm0_{a}$	$^{A}50\pm0_{a}$	$^{A}50\pm0_{a}$	$^{B}40\pm0_{a}$
WH	>70	$^{B}50\pm0_{b}$	$^{C}40\pm0_{b}$	$^{A}>70_{a}$	$^{A}30\pm0_{b}$	$^{A}30\pm0_{b}$	$^{A}27\pm6_{a}$	$^{B}20\pm0_{c}$	$^{A}50\pm0_{a}$	$^{B}20\pm0_{b}$
MD	>70	$^{A}63\pm6_{a}$	$^{B}50\pm0_{a}$	$^{C}30\pm0_{c}$	$^{B}30\pm0_{b}$	$^{A}40\pm0_{a}$	$^{C}20\pm0_{b}$	$^{B}30\pm0_{b}$	$^{A}50\pm0_{a}$	$^{C}20\pm0_{b}$

A-C: Different letters showed significant differences ($p < 0.05$) between drying procedures for each honey and carrier.
a-c: different letters showed significant differences ($p < 0.05$) between carries for each honey and drying procedure.

non-phenolic substances, such as proteins or Maillard reaction products. With the results of all the parameters, a PCA was carried out. The first principal component explained a 34.78% of total observed variance, while the second principal component explained a 22.30% of the total observed variance (Fig. 5A and B). PC1 was mainly defined by TPC, antioxidant and anti-inflammatory activities, while PC2 was mainly defined by methanolic extracts' TPC, flavonoids and antimicrobial activities. LHP could be divided into two different groups. The first group was placed on the right side of the axis and comprised WH-LHP that were the honey powders with higher reducing components, TEAC, ORAC and SRS and lower anti-inflammatory activity. The second group was placed on the left side of the axis and comprised AG-LHP and MD-LHP that were the honey powders with higher AOA and anti-inflammatory activity. LHP1 was placed at the top, showing high flavonoids' contents and the lowest antimicrobial activity (higher MBC). Conversely, LHP3 was placed on the bottom, exhibiting the lowest flavonoids' content and the highest antimicrobial activity. PCA showed no differences among the drying procedures with regard to the studied parameters. Cluster analysis also divided the samples into two groups in a similar way as PCA (Fig. 5C). In the dendogram chart two clusters were observed, the first one with WH-LHP and the second one with AG-LHP and MD-LHP. LHP3 were slightly separated from the other samples in each cluster. Thus, it can be concluded that the botanical species contribution and the type of carrier influences the honey powder's biological properties.

5. Conclusions

All the drying methods and carriers retained the honeys' TPC and flavonoids, although a harmonized procedure to extract honeys' phenolics is necessary for a proper comparison of results. Antioxidant activities of honey powders were higher than those of the raw honeys. WH-LHP showed the highest TEAC, ORAC and SRS, while MD-LHP showed the highest AOA activity. Antioxidant activities of LHP depended on the honey content in the powder, on the carrier used and on the drying procedure. Anti-inflammatory activity of LH and LHP were similar when AG was used as carrier, and decreased if WH or MD were the carriers. Antimicrobial activities of LHP were lower than antimicrobial activities of LH, due to the carrier dilution factor or to the degradation of thermolabile antimicrobial compounds. In general, biological properties of honey powders were mainly influenced by the type of carrier used for dehydration, being barely influenced by the drying procedure.

Declaration of interests

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.

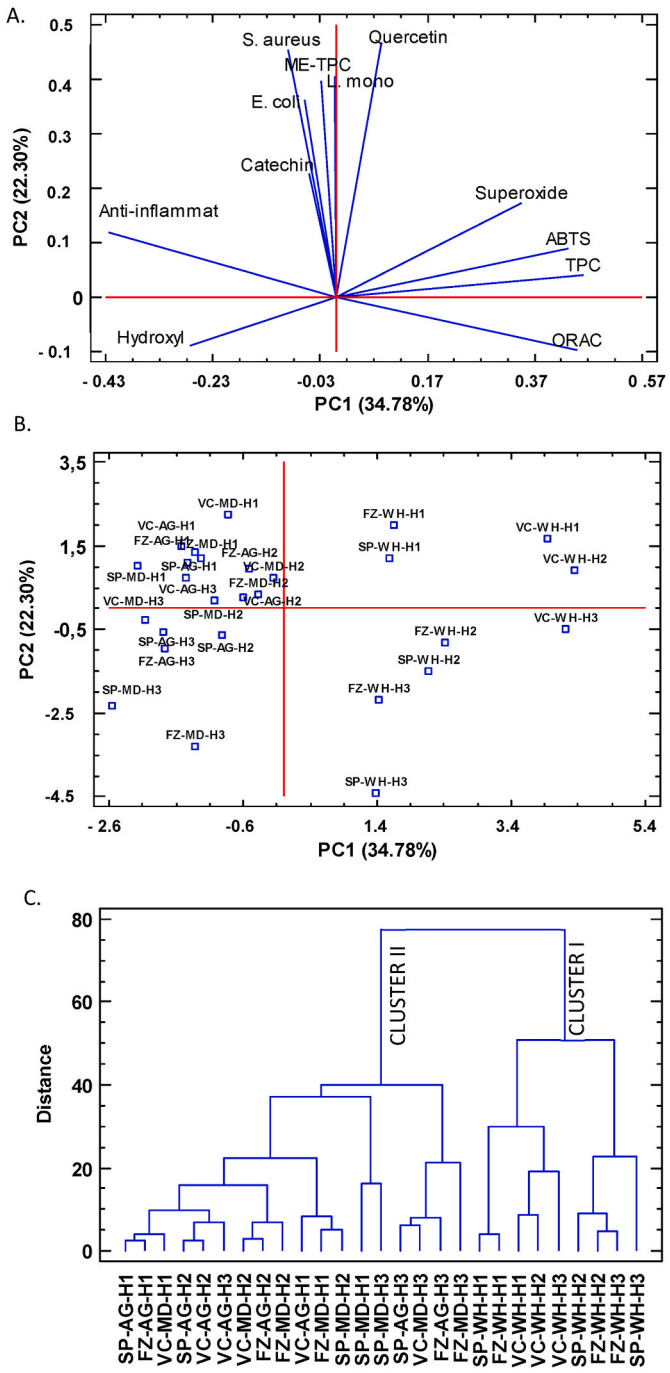

Fig. 5. Loading plot (A) and scores plot (B) of Principal Components Analysis. Dendrogram plot derived from hierarchical cluster analysis (C). ME-TPC: Total phenols compounds in methanolic extracts; SP: Spray drying; VC: vacuum drying; FZ: freeze drying; AG: Arabic gum; WH: whey; MD: maltodextrin; H: honey.

CRediT authorship contribution statement

Sandra María Osés: Conceptualization, Data curation, Investigation, Methodology, Resources, Supervision, Writing – original draft. **Leire Cantero:** Formal analysis, Investigation, Validation. **Guillermo Puertas:** Formal analysis, Investigation, Validation. **Miguel Ángel Fernández-Muiño:** Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft. **María Teresa Sancho:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113235.

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