Physicochemical and biological properties of Spanish *Quercus* honeydew honeys

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Runing title: Spanish oak honeydew honeys

A B S T R A C T

The emergent market for honeydew honey mostly in Europe prompt to increasing requirements of consumers and honey industry for the characterisation of this type of honey. The aim was to characterise a wide sample of Spanish oak honeydew honeys. Physicochemical properties showed values within the limits established by the legislation and typical for honeydew honeys. Darkest samples had the highest levels of total minerals, S, Ca and Fe. Total phenolic (TPC) and flavonoid contents (TFC) showed mean values of 130.25 mg GAE/100 g and 11.30 mg CE/100 g, respectively. Samples showed ability to scavenge radicals ABTS, ranging between 234.64-2252.78 µmoles TE/100 g. All of samples inhibited lipid peroxidation (TBARS values, 10-54%), which is very interesting because, as far as we know, there are no previous studies for honeydew honeys. Results show that our oak honeydew honeys are a source of healthy natural compounds with interest for food industry.

Keywords: Oak honey, Phenols, Flavonoids, Antioxidant activity, TBARS

1. Introduction

Honey is classified according to its botanical sources as either flower honey or honeydew honey. Flower honey derives from the nectar of the flowering plants whereas honeydew honey derives mainly from plant secretions or excretions produced by insects when they feed on plant sap. The honeydew can be produced by a wide variety of sacking insects; it is particularly common as excretion in hemipteran insects, but also in some caterpillars of Lycaenidae butterflies and some moths (Maschwitz, Dumpert, & Tuck, 1986; Delabie, 2001). Several authors have reported the production of honeydew by insects in different European Coniferae such as *Abies*, *Picea*, *Larix* and *Pinus* (Carter & Maslen, 1982; Binazzi & Scheurer, 2009), and also in different *Quercus* species (Persano Oddo & Piro, 2004; Jerković & Marijanović, 2010; Rybak-Chmielewska, Szczęsna, Waś, Jaśkiewicz, & Teper, 2013). In Spain, the main sources of honeydew are holm-oak (*Quercus ilex*), and pyrenean oak (*Q. pyrenaica*), the latter especially in the NW of the country (Castro-Vázquez, Díaz-Maroto, & Pérez-Coello, 2006; Rodríguez Flores, Escuredo, & Seijo, 2015).

In addition, some living parts of plants can also produce themselves secretions, as a result of sores produced by insects or simply by high pressures of phloem. In Spain, these latter secretions are typical in Spanish oak forests during the summer, especially in mountain areas with moderate humidity, where the different oak trees exude a large amount of phloem sap in its acorns. The breaking of the vessels that connect the cupule with the nut of the acorn due to high pressures of phloem can detain acorn growth, which dries up, and falls out. The liberated sweet sap contains natural sugars and minerals and is ingested by bees and deposited in hives as a dark honey (Jerković & Marijanović, 2010; Krakar, 2012).

The composition of honey is rather variable and primarily depends on the floral source, however, certain external factors also play a role such as seasonal and environmental factors and processing. The differences in the chemical composition among honeydew and nectar honey has been indicated in various research (Bentabol Manzanares, Hernández-García, Rodríguez-Galdón, Rodríguez-Rodríguez, & Díaz-Romero, 2011; Simova, Atanassov, Shishiniova, & Bankova, 2012;

Rodríguez Flores et al., 2015; Pita-Calvo & Vázquez, 2017). These studies show that several physicochemical parameters, such as electric conductivity, pH, acidity, ash, and mineral content have generally higher values in honeydew honeys. It is also possible to differentiate honeydew honey from nectar honeys by colour, honeydew honeys were generally characterised by darker than nectar honeys (Vela, De Lorenzo, & Pérez, 2007). However, several researches no found significant differences for moisture and water activity between honeydew and nectar honeys (Bentabol Manzanares et al., 2011). Regarding sugar composition, glucose and fructose are the major carbohydrates and represent about 75% of the sugars found in honey. Sugar composition depends mainly on the honey´s botanical origin, geographical origin and is affected by climate, processing and storage (Escudero, Dobre, Fernández-González, & Seijo, 2014). Honeydew honey has been found to contain higher oligosaccharides contents, mainly trisaccharides such as melezitose and raffinose, as well as lower mean contents of monosaccharides than nectar honey. The concentration of fructose and glucose, as well as the ratio between them, are useful indicators for the classification of monofloral honeys (Bentabol Manzanares et al., 2011).

Honey is known for be part of traditional medicine thanks to its therapeutic properties. These properties are related to antioxidant activity of honey, being phenolic compounds, mainly flavonoids, and minerals very important compounds in this activity. Nowadays, consumers are exhibiting more interest in honeydew honeys than in nectar honeys, which is partially attributed to its better functional properties. Some authors have indicated that honeydew honeys show higher antioxidant activities than nectar honeys and this may be related to the higher values of phenolic compounds in honeydew honey (Vela et al., 2007; Lachman, Orsák, Hejtmánková, & Kovárová, 2010; Escuredo, Míguez, Fernández-González, & Seijo, 2013; Rodríguez Flores et al., 2015).

Despite the functional importance of honeydew honeys, the great amount consumed and the wide geographical production of the honeydew honeys in Spain, studies on the characterisation of this type of honeys are still scarce, except the recent palynological and physicochemical characterisation of the Galician (NW Spain) *Quercus pyrenaica* honeydew honeys (Escudero et al. 2012; Rodríguez Flores et al., 2015).

Therefore, the aim of this work was to evaluate the physicochemical characteristics such as moisture, pH, acidity, electrical conductivity, ash, colour parameters and HMF content of Spanish oak honeydew honeys. In addition, considering the growing interest in honeydew honeys due to their nutritional composition (minerals and sugars) and specially to their antioxidant activity, fourteen mineral elements, nine sugars, total phenolic and flavonoid contents, the ability to scavenge radicals and the lipid peroxidation inhibition were also determinate.

2. Materials and methods

2.1. Honeydew honey samples

The present study examined 59 different oak (*Quercus* spp.) honeydew honey samples collected in 2014 in different provinces of Spain (Fig. 1). These samples come from regions with diverse types of vegetation and were taken directly from professional beekeepers or apicultural associations. The honey samples were aseptic transferred into plastic bottles and stored at 4 ◦C until analyses. All honey samples were certified by the beekeepers as honeydew honeys, showing a dark colour.

2.2. Physicochemical parameters

Physicochemical parameters such as moisture, pH, free, lactonic and total acidity, electrical conductivity, and ash were determined following the Official Methods of Analysis (AOAC, 2005).

The identification and quantification of hydroxymethylfurfural (HMF) was by Ultra Rapid Resolution Liquid Chromatography equipped with a diode-array detector (UHPLC-DAD) following the method described by Jara-Palacios, Hernanz, González-Manzano, Santos-Buelga, Escudero-Gilete, & Heredia (2014). Honey samples were accurately weighed (5 g) dissolved in 10 ml of ultrapure water. Subsequently, 500 µl of honeydew honey sample solution was dissolved in 500 µl of 0.01% formic acid prior to its injection into the UHPLC system. HMF was identified by their retention time and UV-vis spectra by comparison with standard.

2.3. Mineral Contents

Mineral elements were determined using inductively coupled plasma-optical emission spectrophotometer (ICP-OES Horiba Jobin Yvon Ultima 2). The instrumental operating conditions were: RF generator, 1200 W; Frequency of RF generator, 40.68 MHz; Plasma gas flow rate, 15 l/min. The standards solutions of the elements were prepared by diluting stock solution (ICP standard CertiPUR) 1000 mg/l. All dilutions were prepared with deionised water produced by a MilliQ water purification system (Millipore, Belford, USA). Fourteen mineral elements (Al, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, Pb, S, Si and Zn) were determined in each honey. Samples were prepared from exactly 0.4 g put into polytetrafluoroethylene vessels. 7 ml of HNO_3 (PlasmaPURE, SCP, Courtaboeuf, France) and 1 ml of H_2O_2 (suprapure quality, MercK, Darmstadt Germany) were added. The digestion was carried out in a microwave oven (Multiwave 3000, Anton Paar, Austria) with the parameters set for 3 min, 0-850 W at 100 ºC, 10 min 850 W at 170 ºC, 5 min 850 W at 200 ºC, and 15 min ventilation. The resulting solution was brought up to volume 25 ml with deionised water and was subjected to analysis by ICP-OES. An acid blank sample containing the acids used for the digestion was prepared in the same way.

2.4. Sugar Profile

Approximately 1 g of honey was weighed and mixed with 10 ml of 15% acetonitrile. A millilitre of the dissolution was then filtered through a hydrophilic PVDF Millex-HV 0.45 µm syringe filter (Millipore, Bedford, MA, USA) prior to HPLC analysis. HPLC-grade acetonitrile was obtained from Panreac (Barcelona, Spain). Water was purified in a NANOpure® Diamond™ system (Barnsted Inc., Dubuque, IO, USA). Standards of fructose, glucose, sucrose, turanose, maltose, trehalose, isomaltose, melezitose, and raffinose were purchased from Sigma-Aldrich (Madrid, Spain). Stock solutions (5 g/l for glucose, fructose and sucrose; 2 g/l for turanose, maltose, trehalose, isomaltose, melezitose, and raffinose) were prepared for dissolution in ultrapure water and were stored at 4 ºC. The determination of the sugars was performed with an Agilent 1100 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a Differential Refractive Index detector (RID). The separation was performed by using a ZORBAX Carbohydrate Analysis column (4.6 x 250 mm) with a particle-size diameter of 5 μm. The column was maintained at 30 ºC throughout the analysis. The mobile phase was composed of 75% acetonitrile in water. The injection volumes of the samples were 20 μl, with a flow rate of 1.4 ml/min. The HPLC sample peaks were identified by means of comparing the retention times obtained from standards in order to verify the identity of the chromatographic peaks. The quantification of the sugars was carried out by external calibration from the areas of the chromatographic peaks obtained by RID. The corresponding calibration curves were made up of six dilutions of the stock solutions in 15 % acetonitrile for the sugar standards. The limits of detection (LOD) and of quantification (LOQ) were calculated from the calibration curves (Jara-Palacios et al., 2014). The within-laboratory repeatability (within-day precision) was developed in accordance with UNE 82009 standards, and was ascertained by analysing the sugar content in a honeydew honey sample six times within the same day under the same analytical conditions. Within-laboratory reproducibility (day-to-day precision) was assessed by analysing a honeydew honey sample in triplicate over a period of 1 month, whereby the control sample was maintained at 4 ºC. All the samples and standards were injected twice to obtain the averages.

The developed method allows the separation of nine compounds, two monosaccharides (glucose and fructose), five disaccharides (sucrose, turanose, maltose, trehalose and isomaltose) and two trisaccharides (melezitose and raffinose). With respect to the analytical characteristics, all the curves present good linearity ($r^2 > 0.9976$) in the range of concentrations studied. The lowest LOD and LOQ correspond to isomaltose (1.89 mg/l and 6.29 mg/l, respectively) and the highest LOD and

LOQ to fructose (21.33 mg/l and 71.08 mg/l, respectively). Concerning the repeatability, the highest values corresponded to raffinose (4.46%). The highest RSD observed in the reproducibility also corresponded to raffinose (5.08%). Nonetheless, most of the RSD values obtained were below 5.08 %, which confirmed the high reproducibility of the method.

2.5. Antioxidant Activity

Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay with some modifications as previously reported by Kus, Congiu, Teper, Sroka, Jerkovi, & Tuberoso (2014). Gallic acid was employed as a calibration standard and results were expressed as gallic acid equivalents (mg GAE/ 100 g of honey).

Total flavonoid content (TFC) was determined by the aluminum chloride colourimetric method as previously described by Habib, Al Meqbali, Kamal, Souka, & Ibrahim (2014). Honey sample (0.5 ml) was mixed with 2 ml of distilled water and 150 µl of a 5% $NaNO_2$ solution. After 5 min, 150 µl of 10% $AlCl_3$ solution was added and, after 6 min, 2 ml of a 1 mol/l NaOH solution was also added. The final volume was brought to 5 ml with distilled water. Finally, the absorbance was measured at 510 nm and results were expressed as mg catechin equivalent per 100 g of honey (mg CE/100 g).

The antioxidant activity was measured *in vitro* based on the ability to scavenge the $ABTS^{\bullet+}$ radical. The ABTS assay was performed as previously described (Re, Pellegrini, Proteggente, Pannala, Yang, & Rice-Evans, 1999). Honey sample (50 µl) was added to 2 ml of the $ABTS^{\bullet+}$ diluted solution (7 mM ABTS with 2.45 mM potassium persulfate in water) and the absorbance was measured at 734 nm after incubation at room temperature for 4 min. Results were expressed as Trolox equivalent antioxidant capacity (TEAC), considered as the µmol of Trolox with the same antioxidant capacity as 100 g of honey (µmol TE/100 g).

Moreover, the lipid peroxidation inhibition was determined by the TBARS assay, as described by Ohkawa, Ohishi, & Yagi (1979), with some modifications (Jara-Palacios, Escudero-

Gilete, Hernández-Hierro, Heredia, & Hernanz, 2017). Livers of rats were weighed and homogenised in 20 mM Tris-HCl buffer (pH 7.5). The homogenate was centrifuged for 10 min at 3000*g* obtaining a supernatant that was used for the TBARS assay. The reaction mixture, prepared on ice, contained rat liver (200 μl), Tris-HCl buffer (675 μl), honey sample (100 μl), cumene hydroperoxide (20 mM, 25 μl) in a reaction volume of 1 ml. Total oxidation samples contained all reagents except phenolic extracts. The mixture was incubated at 37 °C for 1 h and the reaction was stopped by adding 10% trichloroacetic acid at 4 °C to precipitate the proteins. The mixture was centrifuged at 3000*g* for 10 min and 1 ml of 2-thiobarbituric acid was added to the supernatant, which was incubated at 100 °C for 1 h. The TBARS were measured by determining absorbance at 535 nm. Results are expressed as percentage of inhibition of lipid peroxidation (% inhibition).

2.6. Colour parameters

Colour was assessed by tristimulus from the colourimetry based on reflectance spectra. The spectra have been measured on the honey against a white background, using a CAS-140B spectroradiometer (Instrument System, Munich, Germany). The procedure was carried out as described previously by Terrab, Escudero, González-Miret, & Heredia (2004). The following CIELAB parameters were assessed: L^* (lightness), a^* and b^* (two colour coordinates), h_{ab} (hue angle) and C^*_{ab} (chroma).

2.7. Statistical analysis

Correlations between phenols, flavonoids and minerals content and antioxidant activity (measured by ABTS and TBARS) were studied by both simple and multiple regressions computed by General Linear Models (GLM). The Statistica© v.6.0 software was used for all the statistical treatments.

3. Results and discussion

3.1. Physicochemical parameters

The results of physicochemical parameters (moisture, pH, acidity, hydroxymethylfurfural, electrical conductivity and ash) of honeydew honey are summarised in Table 1. The honey moisture presented values ≤ 20 in all samples with a mean value of 16.5%; this is in agreement with the limit established by the European Community Directive (EU Council, 2002). Similar low levels of water content in Polish honeydew honeys were found by Rybak-Chmielewska et al. (2013). Persano Oddo & Piro (2004) also determined the average water content at 16% in European honeydew honeys.

pH values ranged between 4.34 and 5.14, with a mean value of 4.77. These values agree with those found in Polish *Abies alba* (mean = 4.63) and Galician *Quercus pyrenaica* honeys (mean = 4.4) (Rybak-Chmielewska et al., 2013; Rodríguez Flores et al., 2015). Also, Croatian and Macedonian honeydew honeys present very similar values (mean = 4.8 and 4.7, respectively) (Primorac et al., 2009).

The values for the free acidity ranged between 30.9 and 52.1 meq/kg. According to the EU legislation (EU Council, 2002), the upper limit for free acidity is 50 meq/kg. Only one honey sample (No. 27) exceeded this limit value. High values of free acidity may indicate the fermentation of honey sugar by yeasts. Regarding the lactonic acidity, values ranged between 0.92 and 8.92 meq/kg, while the mean of the total acidity is 43 meq/kg. The results obtained for total acidity are very similar to those found in Macedonian honeydew honeys (mean = 42.6 meq/kg; Primorac et al., 2009), and relatively higher than those found in pine Greek honeys (range: 23.75-44.94 meq/kg; Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014).

Regarding HMF, our honeydew honeys showed very low values of this parameter ranging from 1.32 to 13.41 mg/kg, and none of the honeys exceeded the permitted limit established by the European Community (40 mg/kg). The values obtained for HMF are typical of unprocessed honeys.

According to EU legislation, the lower-limit value of electrical conductivity for honeydew honey is 800 µS/cm. Our results showed values of electrical conductivity ranging from 811 to 1363 µS/cm, with the mean value at 1009 µS/cm. The electrical conductivity values found in our samples are in line with those found in many European honeydew honeys (Turkey: *Quercus robur* and *Pinus* sp.; Poland: *Abies alba*; Greece: *Pinus* sp.; NW Spain: *Quercus pyrenaica*) (Persano Oddo & Piro, 2004; Rybak-Chmielewska et al., 2013; Karabagias et al., 2014; Can, Yildiz, Sahin, Turumtay, Silici, & Kolayli, 2015; Rodríguez Flores et al., 2015).

The ash content in the analysed samples ranged from 0.38 to 1.13%, with a mean value of 0.68%. The ash content is generally used to determine the botanical origin (floral, mixed, or honeydew) of honeys. The values of ash found in this study were similar to those found in pine and fir Greek honeys (Karabagias et al., 2014), but much higher than those found in honeydew honeys from the Soria Province (N Spain) (Nozal Nalda, Bernal Yagüe, Diego Calva, & Martín Gómez, 2005).

3.2. Minerals contents

The concentrations (mg/kg) of the 14 elements quantified in honeydew honey samples are shown in Table 2. The average total mineral content is 2500 mg/kg. The most abundant element in the honeys analysed is potassium, which has an average content of 1845 mg/kg, and accounts for 73% of the total minerals quantified; this finding coincides with those of the majority of authors in the literature, who consider this mineral to be the most abundant in honey. Italian and Polish honeydew honeys have shown a mean content of potassium of 2569 and 2387 mg/kg, respectively (Conti et al., 2007; Madejczyk & Baralkiewicz, 2008).

The second and third most abundant minerals are phosphorus and magnesium, with average values of 211 (8.46%) and 188 mg/kg (7.54%), respectively, while calcium (mean = 106 mg/kg) and sulphur (mean = 87 mg/kg) account for 4.25 and 3.5%, of the total minerals quantified, respectively. Al, Cu, Fe, Ni, Mn, Na, Pb, Si, and Zn, accounts for less than 1% of the total minerals quantified. Several minerals, such as iron and sodium, are present in lower quantities in our samples with respect to honeydew honeys from other regions (mean Fe = 8 mg/kg, in Italian honeys; mean Na = 156 mg/kg, in Colombian honeys), while magnesium and manganese are present in greater quantities with respect to the Polish, Italian, and Anatolian pine honeys (Conti et al., 2007; Madejczyk & Baralkiewicz, 2008; Pisani, Protano, & Riccobono, 2008; Gamboa-Abril, Díaz-Moreno, & Figueroa-Ramírez, 2012; Kaygusuz et al., 2016).

3.3. Sugar Profile

The sugar quantification is summarised in Table 3. The total content of sugars quantified ranged from 63.42 to 73.43 g/100 g, with a mean of 69.16 g/100 g. The content of monosaccharides (the sum of fructose and glucose) ranged from 50.19 to 64.46 g/100 g, with a mean of 57.29 g/100 g. The F/G ratio ranged from 1.14 to 1.55, with a mean of 1.32. The fructose content lay between 30.05 and 37.98 g/100 g, with a mean of 32.58 g/100 g. The glucose content ranged from 19.67 to 27.07 g/100 g, with a mean of 24.71 g/100 g, and this is about 4 to 11 g/100 g lower than the content of fructose. Regarding the disaccharides, the sucrose content varied from 0.01 to 1.31 g/100 g, whereby the maltose was quantitatively the most significant disaccharide, ranging from 2.23 to 6.95 g/100 g (mean = 4.90 g/100 g). The trehalose content ranged from 0.76 to 3.65, and its average was of 2.03 g/100 g. The mean content of the two remaining disaccharides stood at 2.6 g/100 g for turanose, and 1.46 g/100 g for isomaltose. The average value of the total content of disaccharides was 11.17 g/100 g, and varied from 6.33 to 16.63 g/100 g. Regarding the trisaccharide content, the melezitose was quantitatively the most important, ranging from 0.32 to 1.49 g/100 g with a mean of 0.64 g/100 g, while raffinose presented a low mean value of 0.04 g/100 g. The total content of monosaccharides was generally much lower than those found in Macedonian (mean = 70.4 g/100 g), Croatian (mean = 63.3 g/100 g), Polish (mean *Abies alba* = 62 g/100 g), and Turkish honeydew honeys (mean *Quercus* = 65.01 g/100 g, mean *Pinus* = 63.47 g/100 g) (Primorac et al., 2009; Rybak-Chmielewska et al., 2013; Can et al., 2015).

The results from fructose differed only slightly from those of other European countries (Persano Oddo & Piro, 2004), with an average of 32.5 g/100 g, ranging from 28.7 to 36.2 g/100 g, while our values of glucose content remained relatively lower than those found in many European countries (Macedonia: mean glucose content = 36.8 g/100 g; Croatia: mean glucose content = 30.7 g/100 g; Poland: mean glucose content = 27.8 g/100 g). With respect to the fructose/glucose ratio, our results agree with those found: by Persano Oddo & Piro (2004) (mean F/G = 1.25); by Rybak-Chmielewska et al. (2013) (mean F/G = 1.2); and by Golob & Plestenjak (1999) (mean F/G = 1.35), in European, Polish and Slovene honeydew honeys, respectively. Regarding to the disaccharides, it was noticeable that the total content of disaccharides was much higher in these honeydew honeys than in floral honeys. The sucrose content turned out to be much lower than the limit requirements (no more than 5%). Other authors also reported low sucrose content: Szczęsna, Rybak-Chmielewska, & Skubida (2003), and Persano Oddo & Piro (2004), whose average value in their studies was 0.98, 0.2, and 0.8 g/100 g, respectively. Our results concerning maltose content are in agreement with the literature, which ranged from 1.9 to 4.4 % in fir honeydew honey (*Abies alba*) (Rybak-Chmielewska et al., 2013), and from 3.43 to 6.22 % in Macedonian honeydew honey (Primorac et al., 2009). The results of those remaining disaccharides quantified in our study present generally similar values to those of Polish fir honeys, where turanose and trehalose have a mean content of 1.8 and 2.7 g/100 g, respectively (Rybak-Chmielewska et al., 2013). The content of melezitose (a trisaccharide commonly known as larch sugar) is characteristic for honeydew honeys, and is present in honeys made from both deciduous and coniferous honeydew. Similar contents of this trisaccharide were found in *Quercus* (mean = 0.94 g/100 g), *Pinus* (mean = 0.64 g/100 g) and *Abies* (mean = 3.2 g/100 g) honeys (Rybak-Chmielewska et al., 2013; Can et al., 2015). In general, the presence of melezitose in our samples confirms that a substantial part of our honey was made from honeydew.

3.4. Antioxidant activity

Table 4 summarises the antioxidant activity parameters measured in the honey samples. In general terms, all samples presented high contents of total phenols and total flavonoids, with concentrations ranging between 50.04 and 243.86 mg GAE/100 g (average: 130.25 mg GAE/100 g), and between 1.81 and 25.22 mg CE/100 g (average: 11.30 mg CE/100 g), respectively. Concerning total phenols, our results are consistent with those found in Romanian (mean = 127 mg GAE/100 g), Turkish (mean = 120 mg GAE/100 g) and Galician (mean = 132.3 mg GAE/100 g) oak honeys. Regarding total flavonoid content our results are much higher than those found in Turkish oak honeys, but similar to those found in Romanian and Galician honeydew honeys (Al, Daniel, Moise, Bobis, Laslo, & Bogdanov, 2009; Can et al., 2015; Rodríguez Flores et al., 2015).

The antioxidant activity values measured by the ABTS assay ranged from 234.64 to 2252.78 µmoles TE/100 g. Results indicate that honeydew honeys present a high antioxidant activity, which could be related to the high phenolic content (Lachman et al., 2010; Escuredo et al., 2013). A previous study, indicated that the oak honeys had higher total phenolic content and higher antioxidant capacity measured by ABTS method than blossom honeys (Kaygusuz et al., 2016). According literature, phenolic compounds are one of the most important antioxidant compounds found in honey, and the flavonoid content is highly related to the antioxidant activity (Vela et al., 2007; Escuredo et al., 2013); however, this relationship is not confirmed in our study.

On the other hand, honeydew honeys showed inhibition of lipid peroxidation in rat liver homogenates exposed to oxidation. After treatments with honeys, a significant increase ($p<0.01$) in inhibition was observed for all samples. Capacity to inhibit lipid peroxidation measured by TBARS assay ranged between 10.48 and 47.25 % (average: 27.55 %), which indicate a good antioxidant activity in *in vitro* biologic system. Ferreira, Aires, Barreira, & Estevinho (2009) studied the capacity of three varieties of colour honeys (light, ambar and dark) to inhibit the lipid peroxidation in brain tissue from pigs, and concluded that dark honey presented, in all the assays, better antioxidant activity (lower EC50 values) than the other honey samples (ambar and light). As far as we know, no previous studies regarding inhibition of lipid peroxidation of honeydew honeys have been published.

3.5. Colour parameters

Table 1 shows the results obtained for the different colour parameters in the CIELAB colour space. The lightness (L^*) values ranged between 19.59 and 54.57 units. The chroma (C^*_{ab}) values range between 11.87 to 41.37 units (mean = 31.89 units), and regarding the hue (h_{ab}), this parameter ranges between 42.92° to 73.80° in the orange-yellow region. This can be observed graphically in Fig. 2 which shows the projection of the colour points corresponding to each honey sample on the (a^*, b^*)-colour diagram. In addition, in the (a^*, b^*)-colour diagram can be observed that honeydew honeys are classified into two different groups according to the hue. A group of nine samples showed lowest h_{ab} (<55°), coinciding with the most reddish orange honeydew honeys. Moreover, these honeys showed the lowest L^* values (< 34.43 CIELAB units) indicating dark colours. This is in agreement with results obtained by Gonzalez-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia (2005) from honeydew honeys. Dark samples had the highest levels of total minerals, and results showed that the colour parameters, specifically lightness, were greatly correlated with the concentration of some elements such as S, Ca and Fe.

3.6. Statistical analysis

Correlations analysis were applied to explored relationship between the contents of phenolics, flavonoids and minerals and the results of ABTS and TBARS essays. Significant and low linear correlations were found between values of lipid peroxidation inhibition (TBARS) or antioxidant activity by ABTS assay and the contents of phenolic, flavonoids and minerals compounds (p<0.05; R = 0.27 and R = 0.19, respectively). This fact could be because other chemical compounds (enzymes, amino acids, organic acids, Maillard reaction products, ascorbic acid and carotenoids) present in honeys, and not evaluated, influence the antioxidant activity (Alvarez-Suarez et al., 2010; Rodríguez Flores et al., 2015). Although, total phenolics, flavonoids and minerals could be main contributors to antioxidant activity, this activity could depend on a synergistic effect of all the compounds present in honey.

4. Conclusion

In this study the fifty-nine honey samples from different regions of Spain were characterised as honeydew honeys, because to its physicochemical parameters were within the limits established and found in literature. Fourteen minerals and nine sugars were identified in the samples in variable concentrations. Magnesium and manganese are present in greater quantities with respect to the other honeydew honey from other regions, while, total content of monosaccharides was much lower. In a general way, all samples were a rich source of phenolic compounds, among them flavonoids, with great antioxidant activity. In addition, these honeydew honeys showed capacity to protect against lipid peroxidation, which is now reported for the first time. The antioxidant activity of this honeys does not seem to be a property of a single phytochemical compound, but is correlated both to phenolic compounds and minerals. This study could be of great interest for food industry because it shows that honeydew honeys are an important source of healthy natural compounds and have beneficial properties for health, which is much demanded by consumers.

Conflict of interests

The authors declare that they have no conflict of interests.

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<u>Figure legend</u>

Fig. 1. Distribution of the honey samples studied by provinces.

A: Almería; Ab: Albacete; B: Barcelona; Ca, Cádiz; Cc: Cáceres; Cs: Castellón; Cu: Cuenca; Gr: Granada; Gu: Guadalajara; H: Huelva; J: Jaén; M: Madrid; Ma: Málaga; Mu: Murcia; Sa: Salamanca; Z: Zaragoza.

Fig. 2. Distribution of the Spanish oak honeys within CIELAB colour space (*a***b**-diagram).

Table 1

Distribution data for common physicochemical and colour parameters in Spanish oak honeys.

Sample	Moisture (%)	pH	Free acidity (meq/kg)	Lactonic acidity (meq/kg)	Total acidity (meq/kg)	HMF (mg/kg)	Electrical conductivity (µS/cm)	Ash (%)	*L**	*a**	*b**	C^*_{ab}	h_{ab}
1	14.4	4.86	38.4	3.79	42.2	2.11	988	0.62	30.31	18.54	19.86	27.17	46.97
2	16.4	4.71	37.1	3.35	40.5	1.64	950	0.65	53.21	10.31	32.52	34.11	72.40
3	16.6	4.58	44.6	3.79	48.4	13.41	1007	0.76	45.84	12.98	31.61	34.17	67.68
4	18.0	4.76	43.2	3.79	47.0	7.39	1188	0.95	45.19	12.62	30.97	33.44	67.83
5	16.6	4.82	37.0	4.69	41.7	8.00	979	0.65	39.38	14.52	27.19	30.82	61.91
6	14.2	4.61	38.8	3.78	42.6	2.47	850	0.71	52.75	11.78	34.72	36.66	71.26
7	18.4	4.59	34.5	2.81	37.3	3.21	843	0.52	52.75	11.78	34.72	36.66	71.26
8	15.0	4.89	39.1	6.00	45.1	3.73	842	0.67	45.94	11.59	27.12	29.50	66.86
9	15.6	4.80	45.0	8.72	53.7	7.49	1126	0.65	36.76	14.97	26.92	30.80	60.92
10	14.2	4.40	41.2	3.34	44.6	4.00	812	0.72	50.16	13.03	35.17	37.50	69.68
11	15.6	4.53	39.0	4.24	43.2	5.22	811	0.61	52.33	11.35	33.78	35.64	71.43
12	13.4	4.80	33.7	4.23	37.9	5.18	881	0.68	54.57	10.88	33.51	35.23	72.02
13	15.6	4.80	41.3	4.24	45.5	3.24	1041	0.55	51.81	11.27	32.88	34.76	71.08
14	14.0	4.86	38.8	4.23	43.1	2.67	1077	0.76	42.70	14.35	31.62	34.73	65.59
15	13.6	4.65	37.7	3.76	41.5	3.20	818	0.54	54.07	10.31	29.86	31.59	70.94
16	13.4	4.55	39.1	4.66	43.8	4.20	892	0.80	49.06	13.90	37.51	40.00	69.67
17	15.0	4.51	42.3	7.40	49.7	3.44	852	0.51	46.33	10.18	26.04	27.96	68.64
18	16.6	4.34	39.3	5.12	44.4	3.00	846	0.56	49.61	14.29	34.70	37.53	67.62
19	15.4	4.84	45.4	2.89	48.3	4.75	1228	0.74	29.28	16.49	20.31	26.16	50.92
20	14.4	4.76	47.5	5.55	53.0	3.70	1167	0.73	33.84	28.01	29.55	40.72	46.54
21	17.2	5.05	37.4	1.09	38.5	2.89	1104	0.64	36.51	10.95	24.43	26.77	65.85
22	16.4	4.81	33.2	3.33	36.5	2.97	901	0.38	46.19	13.63	35.69	38.20	69.10
23	16.6	5.06	48.0	3.77	51.8	3.68	1363	0.72	38.81	14.58	27.68	31.28	62.22
24	16.2	5.14	32.4	2.45	34.9	3.02	1080	0.68	27.84	14.51	19.29	24.13	53.05
25	20.0	4.96	39.4	3.34	42.7	3.08	1110	0.79	19.59	8.39	8.39	11.87	44.99
26	18.0	4.93	39.7	1.99	41.7	1.32	1099	0.93	22.02	9.56	12.53	15.76	52.68

27	17.2	4.53	52.1	4.70	56.8	5.93	1078	0.85	34.43	30.29	28.17	41.37	42.92
28	15.8	4.68	32.8	3.34	36.2	1.43	828	0.53	53.84	10.08	33.43	34.92	73.21
29	16.4	4.77	30.9	3.34	34.3	3.67	812	1.13	49.36	12.29	36.52	38.53	71.40
30	16.2	4.80	42.7	5.14	47.9	3.15	1232	0.82	34.27	10.20	21.84	24.10	64.96
31	17.8	4.82	43.5	2.89	46.4	1.70	1118	0.74	39.67	12.45	30.30	32.76	67.66
32	15.6	4.87	41.2	3.78	44.9	2.10	1161	0.63	44.35	11.61	31.36	33.44	69.68
33	15.6	4.89	41.3	4.25	45.6	4.93	1197	0.82	41.63	12.57	31.37	33.79	68.16
34	16.8	4.70	39.9	3.31	43.2	3.45	1054	0.65	44.78	12.28	33.61	35.78	69.93
35	16.4	4.75	36.5	3.31	39.9	2.48	1050	0.68	43.20	12.51	33.00	35.29	69.24
36	16.4	4.90	36.9	3.30	40.2	3.17	1062	0.84	45.70	12.17	34.66	36.73	70.65
37	16.4	4.72	35.0	1.39	36.4	2.15	992	0.55	44.43	11.55	32.14	34.15	70.23
38	17.0	4.88	36.5	1.39	37.9	2.27	1088	0.80	43.83	9.73	27.16	28.85	70.29
39	17.0	4.71	41.0	2.34	43.3	3.13	1047	0.72	46.43	10.06	30.79	32.39	71.91
40	15.0	4.70	42.2	1.88	44.1	2.55	1017	0.72	44.54	10.08	29.47	31.14	71.12
41	18.2	4.56	33.7	1.39	35.1	1.74	862	0.55	39.76	14.25	33.32	36.24	66.84
42	15.8	5.01	35.9	1.39	37.3	2.65	1188	0.82	41.46	10.98	31.16	33.04	70.58
43	17.4	4.48	43.1	3.79	46.9	3.38	946	0.72	52.12	7.82	26.92	28.03	73.80
44	15.0	4.57	40.4	3.80	44.2	3.49	909	0.50	40.08	10.45	30.55	32.29	71.11
45	18.6	4.56	31.2	2.34	33.5	2.90	875	0.60	41.21	13.10	33.28	35.76	68.51
46	18.8	4.35	49.2	3.79	53.0	5.40	915	0.43	36.94	14.09	31.35	34.37	65.79
47	16.8	4.84	35.7	3.31	39.0	2.82	1064	0.62	35.57	12.24	29.00	31.47	67.11
48	16.8	4.43	42.1	3.30	45.4	2.19	850	0.51	43.81	12.66	35.79	37.97	70.52
49	18.2	4.65	39.8	2.83	42.7	1.90	1019	0.59	34.46	11.37	27.45	29.72	67.50
50	17.2	4.88	36.9	1.87	38.8	1.56	1064	0.67	30.77	8.20	9.88	12.84	50.30
51	18.2	4.90	37.5	1.87	39.4	1.92	1159	0.63	32.38	12.37	27.78	30.41	65.99
52	17.0	4.86	36.5	2.83	39.3	2.48	1110	0.68	37.38	13.41	33.32	35.92	68.07
53	17.4	4.53	42.7	4.27	47.0	1.89	908	0.41	38.41	13.63	33.02	35.72	67.57
54	19.4	4.69	39.3	1.87	41.2	2.08	908	0.67	39.24	12.48	34.02	36.24	69.86
55	17.4	4.89	39.8	0.92	40.7	1.85	1032	0.81	24.95	12.68	13.23	18.32	46.21
56	19.0	4.67	44.6	3.32	47.9	1.87	1031	0.70	35.18	15.11	25.82	29.92	59.66
57	18.2	4.90	40.4	2.84	43.2	1.81	1172	0.77	35.75	11.81	23.85	26.61	63.66
58	17.0	4.73	39.4	2.83	42.2	1.98	894	0.59	39.25	13.80	27.62	30.87	63.45
59	18.0	4.76	39.5	2.84	42.3	1.41	1048	0.67	39.61	15.44	30.71	34.38	63.31

Mean	16.5	4.74	39.5	3.43	43.0	3.33	1009	0.68	41.08	12.88	28.89	31.89	65.15
SD	1.5	0.18	4.34	1.42	5.07	2.00	132	0.13	8.12	3.71	6.42	6.16	7.99
Min	13.4	4.34	30.9	0.92	33.6	1.32	811	0.38	19.59	7.82	8.39	11.87	42.92
Max	20.0	5.14	52.1	8.72	56.8	13.41	1363	1.13	54.57	30.29	37.51	41.37	73.80

SD. Standard deviation.

Table 2

Distribution data for mineral content (mg/kg) in Spanish oak honeys.

Sample	Al	Ca	Cu	Fe	K	Li	Mg	Mn	Na	P	Pb	S	Si	Zn
1	1.28	105	1.44	0.71	1831	0.64	234	24.4	12.9	218	≤ 0.70	104	7.22	≤ 0.08
2	4.06	90.5	1.10	0.85	1649	1.01	175	17.3	16.1	220	≤ 0.70	96.3	18.1	≤ 0.08
3	≤ 0.08	59.2	1.21	0.72	1772	0.64	197	17.3	15.5	220	≤ 0.70	89.9	4.07	≤ 0.08
4	≤ 0.08	90.2	1.33	0.37	2163	0.75	213	15.9	21.4	266	≤ 0.70	109	8.98	≤ 0.08
5	0.71	124	1.20	0.79	1764	≤ 0.23	181	25.6	31.2	165	≤ 0.70	89.6	10.4	≤ 0.08
6	≤ 0.08	81.4	0.71	0.25	1529	≤ 0.23	157	5.70	11.2	173	≤ 0.70	68.8	4.11	≤ 0.08
8	0.12	106	0.95	0.18	1969	0.85	256	19.1	8.40	240	≤ 0.70	89.7	8.90	≤ 0.08
9	≤ 0.08	84.3	1.08	0.14	2056	0.45	255	26.0	14.5	227	≤ 0.70	94.7	3.27	≤ 0.08
10	1.11	56.0	0.78	4.18	1449	≤ 0.23	91	8.52	24.4	218	≤ 0.70	53.8	8.54	≤ 0.08
11	0.99	69.4	1.08	1.63	1422	≤ 0.23	112	8.04	29.6	197	≤ 0.70	69.3	4.27	≤ 0.08
12	1.84	57.0	1.12	1.25	1682	≤ 0.23	93	5.07	11.5	223	≤ 0.70	54.1	3.85	≤ 0.08
13	1.52	181	1.19	1.13	2020	≤ 0.23	169	15.0	26.0	235	≤ 0.70	104	9.85	≤ 0.08
14	≤ 0.08	77.9	1.56	5.05	2021	≤ 0.23	198	17.1	22.6	256	≤ 0.70	96.3	9.95	≤ 0.08
15	1.08	194	0.71	1.99	1448	≤ 0.23	143	8.46	17.7	177	≤ 0.70	88.5	8.95	≤ 0.08
16	0.39	80.5	0.93	1.36	1559	≤ 0.23	114	9.42	32.9	191	≤ 0.70	67.0	10.0	≤ 0.08
17	≤ 0.08	102	0.97	1.98	1541	≤ 0.23	145	13.8	26.3	197	≤ 0.70	86.7	3.80	≤ 0.08
18	≤ 0.08	75.8	0.68	0.62	1391	≤ 0.23	72	3.99	32.0	233	≤ 0.70	41.3	4.66	≤ 0.08
19	0.19	195	1.24	1.02	2196	0.57	253	25.1	23.6	248	≤ 0.70	127	7.88	≤ 0.08
20	3.68	132	1.34	2.50	2256	≤ 0.23	238	22.4	21.9	250	≤ 0.70	93.8	7.34	≤ 0.08
21	0.77	193	1.26	3.83	1975	0.53	272	40.6	14.2	249	≤ 0.70	114	4.68	≤ 0.08
22	0.96	124	1.35	1.25	1500	≤ 0.23	109	5.24	14.9	216	≤ 0.70	53.6	3.64	≤ 0.08
23	3.02	211	1.68	2.27	2428	0.40	327	23.1	16.8	245	≤ 0.70	129	9.33	≤ 0.08
24	1.50	167	1.06	0.51	1736	0.61	270	26.3	9.23	222	≤ 0.70	96.7	7.59	≤ 0.08
25	≤ 0.08	145	1.09	≤ 0.07	1961	0.63	295	33.7	8.75	227	≤ 0.70	99.4	3.16	≤ 0.08
26	1.07	179	1.21	0.95	1991	0.52	292	33.0	13.9	218	≤ 0.70	102	4.05	≤ 0.08
27	0.11	169	3.14	1.26	1998	≤ 0.23	193	61.0	14.9	217	≤ 0.70	111	3.47	≤ 0.08
28	0.85	119	1.00	1.76	1545	≤ 0.23	149	11.4	13.4	195	≤ 0.70	80.8	7.68	≤ 0.08
29	1.88	135	1.16	1.23	1535	≤ 0.23	64	0.31	14.0	186	≤ 0.70	42.6	3.14	≤ 0.08
30	0.59	159	0.79	2.18	2185	0.48	285	27.0	24.2	252	≤ 0.70	120	10.1	≤ 0.08
31	≤ 0.08	145	1.22	0.55	2015	≤ 0.23	221	55.6	15.7	218	≤ 0.70	102	7.12	≤ 0.08
32	2.07	114	1.25	1.24	2202	≤ 0.23	237	22.9	15.2	236	≤ 0.70	109	9.75	≤ 0.08
33	4.89	72.8	1.27	1.28	2238	≤ 0.23	246	21.0	14.8	237	≤ 0.70	103	10.2	≤ 0.08
34	0.80	124	1.37	2.56	1969	0.51	188	12.9	25.8	209	≤ 0.70	84.1	8.45	≤ 0.08

35	≤ 0.08	45.1	1.16	0.75	1960	≤ 0.23	196	13.9	22.4	211	≤ 0.70	87.1	3.82	≤ 0.08
36	2.55	77.1	1.11	3.72	2056	≤ 0.23	212	12.7	16.0	224	≤ 0.70	80.9	8.96	≤ 0.08
37	≤ 0.08	79.0	1.39	0.58	1812	≤ 0.23	182	37.5	27.4	151	≤ 0.70	79.2	7.06	≤ 0.08
38	≤ 0.08	117	1.08	2.15	2112	≤ 0.23	218	23.2	13.8	217	≤ 0.70	84.9	7.26	≤ 0.08
39	≤ 0.08	70.3	1.21	5.07	1946	0.45	202	29.0	20.3	223	≤ 0.70	82.4	7.52	≤ 0.08
40	≤ 0.08	81.7	1.19	4.25	1877	0.49	193	25.9	19.4	224	≤ 0.70	80.5	7.91	≤ 0.08
41	≤ 0.08	32.9	1.31	0.04	1458	≤ 0.23	77	21.8	38.4	111	≤ 0.70	78.4	16.0	≤ 0.08
42	0.69	66.0	1.05	3.05	2318	0.39	232	17.7	14.4	209	≤ 0.70	82.8	6.42	≤ 0.08
43	2.02	209	1.35	11.96	1693	0.46	152	17.0	28.1	218	≤ 0.70	87.6	9.04	≤ 0.08
44	≤ 0.08	57.1	0.96	2.14	1693	≤ 0.23	175	20.2	31.3	189	≤ 0.70	77.7	6.28	≤ 0.08
45	≤ 0.08	25.1	1.25	0.59	1390	≤ 0.23	74	21.2	35.5	105	≤ 0.70	76.9	13.7	≤ 0.08
46	2.02	55.1	1.05	1.06	1610	≤ 0.23	145	12.4	39.5	186	≤ 0.70	87.9	7.53	≤ 0.08
47	≤ 0.08	94.5	1.64	1.04	1982	≤ 0.23	206	33.2	30.5	215	≤ 0.70	85.8	8.93	≤ 0.08
48	≤ 0.08	64.1	0.61	0.75	1677	0.41	151	7.07	16.8	182	≤ 0.70	72.5	3.67	≤ 0.08
49	≤ 0.08	65.6	1.11	5.93	1857	0.83	171	14.3	31.0	230	≤ 0.70	77.4	8.46	≤ 0.08
50	≤ 0.08	97.0	1.31	7.17	1962	≤ 0.23	184	60.7	19.3	196	≤ 0.70	111	11.5	≤ 0.08
51	≤ 0.08	85.5	1.39	1.07	2084	≤ 0.23	225	37.7	15.5	204	≤ 0.70	72.9	11.0	≤ 0.08
52	≤ 0.08	88.1	1.19	0.67	2090	0.45	217	38.3	18.0	182	≤ 0.70	89.8	8.64	≤ 0.08
53	0.14	36.7	1.53	0.79	1518	0.43	172	14.6	38.1	177	≤ 0.70	83.2	10.3	≤ 0.08
54	3.02	64.4	2.61	1.30	1619	≤ 0.23	127	8.02	26.0	231	≤ 0.70	63.4	27.1	≤ 0.08
55	≤ 0.08	95.0	1.21	2.53	1901	≤ 0.23	186	54.5	16.7	220	≤ 0.70	103	6.89	≤ 0.08
56	≤ 0.08	80.4	1.33	0.98	1849	0.59	212	19.0	19.6	217	≤ 0.70	100	7.06	≤ 0.08
57	≤ 0.08	124	1.41	1.97	2129	≤ 0.23	247	52.6	11.7	221	≤ 0.70	108	6.91	≤ 0.08
58	≤ 0.08	65.6	0.99	0.20	1574	0.55	153	39.3	19.0	188	≤ 0.70	77.9	4.29	≤ 0.08
59	1.11	282	1.29	1.58	1871	≤ 0.23	163	9.46	28.3	215	≤ 0.70	58.9	8.68	≤ 0.08
Mean	0.85	106	1.23	1.91	1845	0.37	188	22.5	20.9	211	0.70	87.3	7.96	0.08
SD	1.13	52.3	0.39	2.03	267	0.20	60.5	14.4	8.01	30.8	-	19.1	4.01	-
Min	0.08	25.1	0.61	0.04	1390	0.23	64.0	0.31	8.40	105	-	41.3	3.14	-
Max	4.89	282	3.14	12.0	2428	1.01	327	61	39.5	266	-	129	27.1	-
%[a]	0.03	4.25	0.05	0.08	73.98	0.01	7.54	0.90	0.84	8.46	0.03	3.50	0.32	0.003

[a]: percentage content of each mineral of the total mineral quantified

Table 3

Table 3

Sugar content in Spanish oak honeys (g/100 g).

	Monosaccharide%		Disaccharide%					Trisaccharide%		F+G	F/G	ΣSugars[a]
Sample	Fructose	Glucose	Sucrose	Turanose	Maltose	Trehalose	Isomaltose	Melezitose	Raffinose			
1	32.85±0.48	24.42±0.39	0.23±0.01	2.56±0.04	4.03±0.22	0.88±0.11	1.20±0.03	1.15±0.14	0.05±0.01	57.28	1.24	67.40
2	31.19±0.23	25.21±0.26	0.20±0.04	2.15±0.11	3.42±0.08	0.87±0.10	0.99±0.03	1.46±0.16	0.03±0.01	56.41	1.33	65.56
3	31.47±0.42	23.70±0.44	0.09±0.01	2.29±0.01	4.07±0.02	0.76±0.04	0.79±0.04	1.37±0.04	0.05±0.01	55.19	1.24	64.64
4	30.42±0.13	24.47±0.59	0.10±0.01	2.38±0.05	4.34±0.04	1.01±0.12	1.03±0.03	1.37±0.14	0.01±0.01	54.91	1.43	65.17
5	37.97±0.90	26.48±0.80	0.16±0.04	2.30±0.02	3.69±0.16	0.79±0.04	1.03±0.01	0.49±0.01	0.01±0.01	64.46	1.34	72.95
6	34.43±0.60	25.76±0.92	0.25±0.02	2.17±0.17	3.58±0.34	0.84±0.04	1.26±0.08	0.92±0.03	0.03±0.01	60.20	1.43	69.27
7	34.40±0.46	24.08±0.63	0.46±0.04	2.13±0.03	2.23±0.08	0.79±0.01	0.72±0.03	0.88±0.07	0.03±0.01	58.48	1.32	65.75
8	31.88±0.41	24.23±0.91	0.39±0.01	2.34±0.12	4.47±0.03	0.95±0.04	1.48±0.14	0.74±0.04	0.02±0.01	56.11	1.40	66.57
9	32.79±0.41	23.49±0.38	0.09±0.02	2.65±0.07	5.47±0.19	1.42±0.09	1.28±0.02	0.36±0.03	0.02±0.01	56.29	1.33	67.62
10	36.02±0.11	27.07±0.59	0.02±0.01	2.47±0.14	4.43±0.13	1.76±0.05	1.25±0.01	0.34±0.01	0.04±0.01	63.10	1.37	73.43
11	34.63±0.69	25.20±0.27	0.06±0.01	2.44±0.09	5.41±0.01	1.64±0.08	1.25±0.07	0.40±0.03	0.02±0.01	59.84	1.34	71.10
12	33.45±0.38	24.90±0.48	0.13±0.05	2.89±0.24	5.09±0.06	1.35±0.05	1.61±0.06	0.64±0.01	0.03±0.01	58.36	1.35	70.12
13	33.94±0.27	25.16±0.35	0.22±0.05	2.33±0.06	5.53±0.24	2.14±0.20	1.46±0.25	0.46±0.01	0.05±0.01	59.11	1.40	71.30
14	32.90±0.42	23.44±0.08	0.31±0.04	2.73±0.13	6.18±0.18	2.53±0.02	1.90±0.26	0.45±0.07	0.01±0.01	56.35	1.35	70.48
15	34.48±0.10	25.63±0.22	0.07±0.01	2.21±0.04	5.07±0.12	1.75±0.17	1.06±0.01	0.62±0.05	0.03±0.01	60.11	1.33	70.95
16	34.15±0.42	25.66±0.51	0.01±0.01	2.73±0.14	5.32±0.01	2.35±0.02	1.52±0.30	0.59±0.05	0.07±0.01	59.82	1.37	72.43
17	33.18±0.53	24.19±0.20	0.07±0.02	2.64±0.02	6.65±0.16	2.02±0.01	1.33±0.10	0.49±0.01	0.04±0.01	57.38	1.35	70.63
18	34.79±0.23	25.74±0.54	0.02±0.01	2.57±0.05	5.10±0.48	2.56±0.04	1.20±0.05	0.63±0.05	0.03±0.01	60.53	1.30	72.66
19	33.75±0.13	25.87±1.61	0.23±0.01	1.97±0.05	6.06±0.27	1.70±0.13	1.12±0.14	0.64±0.06	0.01±0.01	59.63	1.47	71.37
20	32.09±0.01	21.78±0.44	0.01±0.01	2.99±0.09	6.91±0.26	3.65±0.09	3.07±0.12	1.49±0.01	0.05±0.01	53.88	1.26	72.05
21	32.27±1.08	25.63±1.46	0.65±0.01	2.66±0.10	3.36±0.10	2.02±0.09	1.15±0.01	0.91±0.07	0.01±0.01	57.91	1.48	68.68
22	33.31±0.28	22.58±0.56	0.22±0.01	3.25±0.10	6.32±0.25	1.86±0.08	2.00±0.07	0.98±0.04	0.05±0.01	55.90	1.34	70.60
23	31.88±0.06	23.75±0.62	0.18±0.01	2.58±0.24	4.60±0.31	2.22±0.03	1.78±0.16	0.60±0.13	0.03±0.01	55.64	1.27	67.66
24	31.39±0.31	24.65±0.93	1.31±0.02	2.99±0.08	3.87±0.20	1.34±0.01	1.49±0.01	1.08±0.03	0.09±0.01	56.05	1.14	68.24
25	30.73±0.09	26.85±0.66	0.10±0.01	2.71±0.05	3.10±0.22	1.68±0.14	1.28±0.09	1.19±0.17	0.03±0.01	57.59	1.18	67.69
26	30.13±0.35	25.53±0.51	0.19±0.02	3.16±0.03	3.82±0.05	1.49±0.09	0.62±0.66	1.14±0.02	0.02±0.01	55.68	1.55	66.13
27	30.51±0.63	19.66±0.60	0.12±0.01	2.98±0.03	6.78±0.11	3.15±0.35	3.19±0.45	0.42±0.01	0.02±0.01	50.19	1.29	66.86
28	33.46±0.23	25.96±0.23	0.40±0.07	2.18±0.07	4.06±0.11	1.60±0.10	1.01±0.03	0.84±0.06	0.02±0.01	59.43	1.45	69.56
29	33.02±0.17	22.73±0.07	0.01±0.01	2.89±0.11	6.95±0.22	2.98±0.07	2.78±0.24	0.40±0.01	0.02±0.01	55.76	1.37	71.79
30	32.60±0.26	23.80±0.62	0.21±0.01	2.68±0.01	4.81±0.13	1.66±0.02	1.45±0.01	0.70±0.01	0.01±0.01	56.41	1.30	67.96
31	33.34±0.42	25.61±0.28	0.03±0.01	2.50±0.08	4.77±0.23	1.76±0.06	1.31±0.04	0.49±0.01	0.02±0.01	58.96	1.35	69.85
32	33.03±0.36	24.49±0.20	0.27±0.01	2.60±0.02	5.59±0.18	2.18±0.18	1.52±0.02	0.52±0.01	0.04±0.01	57.53	1.36	70.27

33	32.34±0.04	23.80±0.06	0.13±0.01	2.62±0.05	5.19±0.10	2.01±0.01	1.48±0.05	0.72±0.02	0.01±0.01	56.14	1.35	68.32
34	31.54±0.13	23.35±0.10	0.03±0.01	2.87±0.13	6.17±0.03	2.24±0.42	1.50±0.05	0.43±0.01	0.02±0.01	54.90	1.38	68.18
35	32.46±0.01	23.60±0.24	0.09±0.03	3.02±0.14	6.40±0.26	2.58±0.03	1.71±0.25	0.43±0.03	0.04±0.01	56.07	1.30	70.35
36	31.75±0.30	24.34±0.08	0.34±0.02	2.76±0.10	5.46±0.01	2.46±0.04	1.47±0.19	0.37±0.01	0.06±0.01	56.09	1.31	69.02
37	30.04±0.20	22.89±0.13	0.12±0.02	2.58±0.32	4.33±0.32	1.86±0.07	1.15±0.26	0.38±0.02	0.04±0.01	52.94	1.28	63.42
38	31.88±0.26	24.87±0.05	0.11±0.01	2.53±0.03	4.41±0.10	2.33±0.01	1.29±0.18	0.51±0.01	0.07±0.01	56.76	1.26	68.02
39	32.24±0.18	25.60±0.49	0.08±0.04	2.49±0.05	4.52±0.01	2.24±0.20	1.15±0.01	0.44±0.03	0.07±0.01	57.84	1.27	68.84
40	32.10±0.09	25.18±0.37	0.05±0.01	2.47±0.18	4.81±0.24	2.13±0.17	1.48±0.08	0.47±0.05	0.09±0.01	57.29	1.36	68.81
41	34.56±0.14	25.39±0.03	0.01±0.01	2.19±0.12	4.17±0.03	2.08±0.27	1.38±0.06	0.35±0.01	0.04±0.01	59.96	1.34	70.20
42	32.14±0.13	24.07±0.10	0.42±0.03	2.78±0.30	4.82±0.19	2.46±0.11	1.57±0.05	0.41±0.01	0.05±0.01	56.21	1.28	68.75
43	33.14±0.02	25.86±0.46	0.01±0.01	2.73±0.19	4.76±0.14	2.73±0.04	1.81±0.11	0.40±0.03	0.08±0.01	59.01	1.36	71.53
44	32.70±0.17	24.08±0.04	0.20±0.01	2.71±0.13	5.56±0.05	2.56±0.13	1.55±0.01	0.41±0.04	0.01±0.01	56.78	1.32	69.80
45	34.02±0.05	25.75±0.16	0.16±0.01	2.56±0.09	4.27±0.01	2.22±0.37	1.41±0.30	0.35±0.01	0.01±0.01	59.78	1.26	70.78
46	31.96±0.11	25.32±0.36	0.02±0.01	2.43±0.08	4.82±0.01	2.36±0.01	1.45±0.04	1.49±0.18	0.07±0.01	57.28	1.30	69.92
47	33.09±0.37	25.40±0.21	0.20±0.06	2.80±0.03	4.63±0.05	2.53±0.14	1.80±0.09	0.38±0.01	0.08±0.01	58.49	1.31	70.94
48	31.93±0.06	24.31±0.45	0.06±0.01	2.83±0.03	5.97±0.33	2.40±0.01	1.56±0.15	0.53±0.01	0.05±0.01	56.24	1.33	69.67
49	31.60±0.73	23.80±0.06	0.15±0.01	2.88±0.11	5.74±0.21	2.83±0.02	2.05±0.08	0.38±0.02	0.02±0.01	55.41	1.26	69.48
50	32.49±0.17	25.81±0.18	0.12±0.03	2.49±0.11	3.92±0.14	2.23±0.03	1.19±0.05	0.39±0.01	0.02±0.01	58.31	1.30	68.67
51	32.72±0.21	25.24±0.35	0.22±0.09	2.90±0.11	4.54±0.23	2.38±0.01	1.43±0.03	0.37±0.03	0.02±0.01	57.97	1.27	69.85
52	31.09±0.13	24.41±0.21	0.18±0.07	2.99±0.05	5.59±0.20	2.58±0.01	1.38±0.14	0.39±0.01	0.02±0.01	55.51	1.22	68.66
53	30.98±0.22	25.41±0.30	0.06±0.01	2.37±0.42	4.91±0.21	2.05±0.07	1.01±0.04	0.36±0.01	0.10±0.01	56.40	1.22	67.27
54	31.37±0.03	25.76±0.63	0.03±0.01	2.60±0.02	4.23±0.01	2.44±0.05	1.28±0.10	0.32±0.01	0.02±0.01	57.13	1.19	68.07
55	31.38±0.28	26.45±0.01	0.20±0.01	2.51±0.01	4.18±0.17	2.30±0.06	1.12±0.08	0.41±0.03	0.01±0.01	57.83	1.23	68.58
56	30.09±0.23	24.53±0.25	0.01±0.01	2.35±0.09	4.85±0.23	2.32±0.14	1.53±0.08	0.82±0.03	0.05±0.01	54.62	1.27	66.57
57	31.22±0.15	24.55±0.19	0.22±0.03	2.55±0.10	5.47±0.32	2.50±0.07	1.45±0.18	0.35±0.02	0.10±0.01	55.78	1.23	68.42
58	32.69±0.32	26.52±0.12	0.08±0.03	2.47±0.12	4.41±0.01	2.34±0.04	1.44±0.11	0.45±0.01	0.01±0.01	59.21	1.32	70.42
59	31.83±0.08	24.07±0.32	0.15±0.01	2.86±0.01	5.87±0.09	3.17±0.21	2.59±0.24	0.48±0.04	0.03±0.01	55.91	1.24	71.07
Mean±SD	32.58±1.48	24.71±1.29	0.17±0.20	2.60±0.27	4.90±0.99	2.03±0.64	1.46±0.48	0.64±0.33	0.04±0.02	57.29±2.30	1.32±0.08	69.16±2.12
Min	30.05	19.67	0.01	1.97	2.23	0.76	0.62	0.32	0.01	50.19	1.14	63.42
Max	37.98	27.07	1.31	3.25	6.95	3.65	3.19	1.49	0.1	64.46	1.55	73.43

[a]: sum of all sugars quantified in each honey sample. F: fructose. G: glucose. SD: standard deviation.

Table 4

Distribution data for total phenolic content (mg GAE/100 g), total flavonoid content (mg CE/100 g), and antioxidant activity (ABTS and TBRAS assays: µmol TE/100 g and % inhibition, respectively) in Spanish oak honeys.

Sample	TPC	TFC	ABTS	TBARS
1	133.52	11.99	527.60	51.41
2	187.51	5.50	775.74	36.35
3	106.51	12.41	838.91	53.17
4	179.30	14.10	585.87	34.79
5	104.52	8.74	632.59	41.52
6	125.34	4.86	651.68	45.78
7	155.80	7.71	1407.93	16.83
8	131.92	7.14	694.06	53.38
9	140.61	8.93	892.37	22.98
10	82.23	11.73	863.78	46.27
11	135.59	13.70	598.42	12.12
12	132.47	12.30	555.19	32.65
13	111.71	22.97	556.19	11.23
14	78.76	14.93	1804.35	20.22
15	110.40	10.57	731.59	14.67
16	106.10	8.94	817.93	24.84
17	57.38	12.61	1244.17	21.20
18	112.41	7.80	490.96	20.67
19	191.39	11.30	1309.58	20.54
20	161.75	17.77	978.77	29.96
21	243.26	13.95	863.63	20.61
22	222.84	8.69	567.58	28.70
23	177.13	17.96	936.13	20.20
24	156.50	14.69	854.38	24.21
25	208.56	23.00	1513.28	10.49
26	103.67	15.21	1286.74	23.05
27	88.29	25.22	878.22	26.54
28	60.45	11.66	2252.78	30.18
29	55.19	4.82	674.01	26.02
30	92.21	13.97	674.34	22.33
31	100.05	12.79	1014.65	29.47
32	80.22	11.06	903.61	17.77
33	87.59	9.16	657.39	22.33
34	120.82	8.33	954.67	23.88
35	93.68	15.89	470.23	18.51
36	50.04	2.93	524.21	14.89
37	99.82	3.57	1028.64	16.35
38	182.25	8.72	789.42	15.09
39	105.46	11.25	1104.34	17.76
40	116.19	13.46	861.63	26.75
41	210.04	8.60	1083.25	35.19
42	124.74	7.28	619.78	23.26

43	223.44	11.05	795.26	21.69
44	243.86	11.63	634.85	26.96
45	200.30	11.77	1269.06	16.95
46	210.11	12.73	799.86	23.19
47	171.34	12.08	568.40	21.15
48	61.17	9.87	605.89	20.48
49	106.29	8.80	457.76	20.33
50	106.18	8.79	1198.83	20.19
51	86.63	1.81	1069.39	52.98
52	104.77	9.11	234.65	41.27
53	121.31	10.25	827.61	48.93
54	125.40	8.63	392.57	42.46
55	92.37	12.48	1260.21	33.93
56	152.97	14.87	653.28	39.73
57	133.49	12.05	1118.75	22.38
58	113.72	11.89	787.60	38.43
59	107.43	10.50	522.81	30.41
Mean	130.25	11.30	858.77	27.55
SD	49.26	4.46	353.23	11.43
Min	50.04	1.81	234.65	10.49
Max	243.86	25.22	2252.78	53.38

Figure 1

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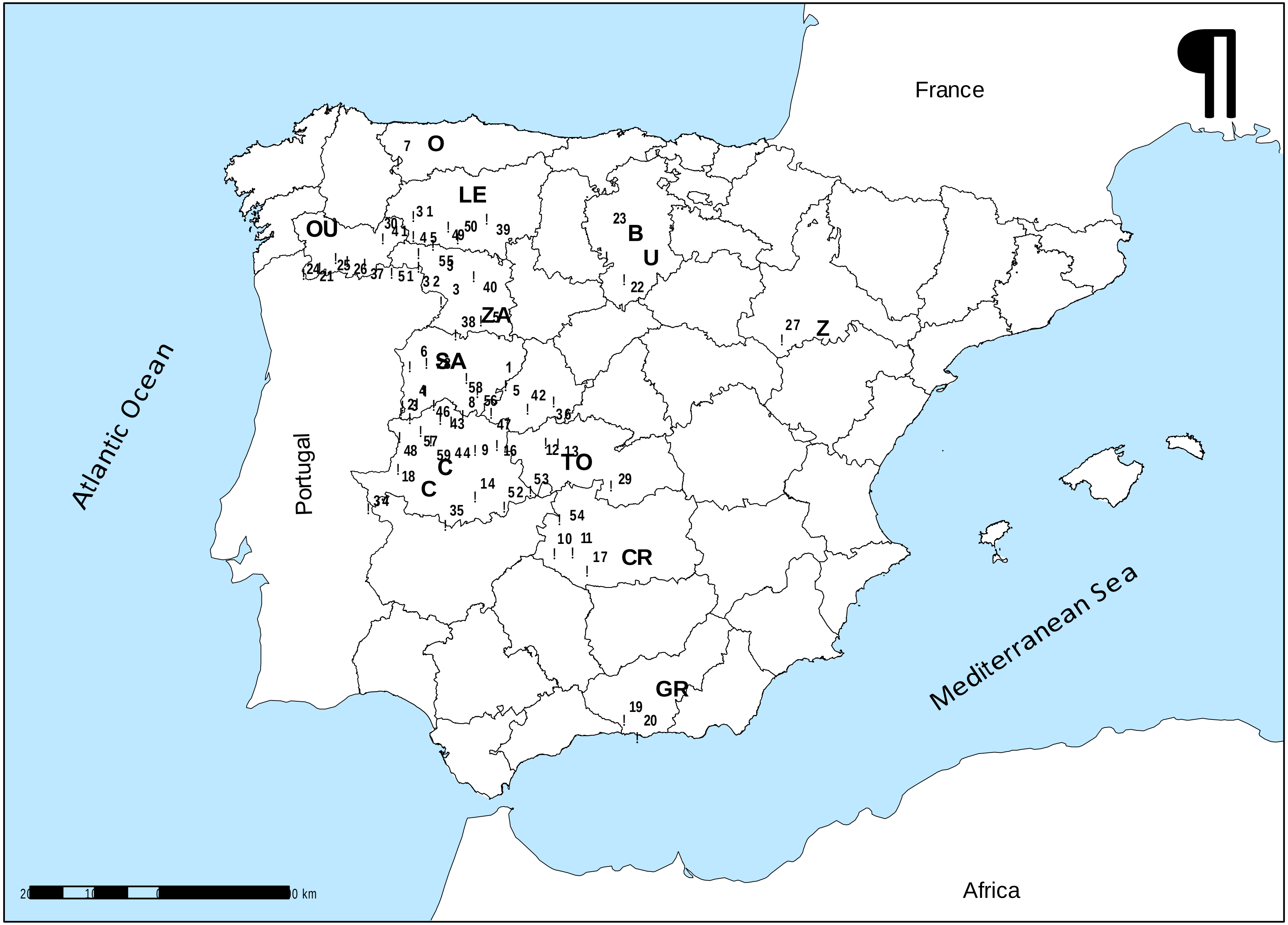

Figure 2

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