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ORIGINAL PAPER

Physicochemical properties, colour, chemical composition, and antioxidant activity of Spanish *Quercus* honeydew honeys

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Abstract

The emergent market for honeydew honeys in Europe prompt to increasing requirements of consumers and honey industry for the characterisation of this type of honey. The aim of this study was to characterise 59 samples of Spanish oak honeydew honeys. Physicochemical properties showed values within the limits established by the legislation and typical for honeydew honeys. Honeys were differentiated into two groups according to the hue (h_{ab}) and all were classified as dark honeys ($L^* < 55$). A total of 14 minerals were determined, with K, P, Mg, and Ca being the most abundant. The development and validation of an HPLC method allowed the determination of the contents of two monosaccharides, five disaccharides, and two trisaccharides. Total phenolic and flavonoid contents showed mean values of 130.2 mg/100 g and 11.3 mg/100 g of honey, respectively. Honeydew honeys showed ability to scavenge free radicals and to inhibit lipid peroxidation, which is very interesting, because, as far as we know, there are no previous studies for this type of honey. Results showed that all honeydew honeys are a source of chemical compounds with nutritional and antioxidant properties that could be of interest for consumers and food industry.

Keywords Oak honey · Minerals · Sugars · Phenols · Flavonoids · Antioxidant activity

Abbreviations

CE	Catechin equivalents
GAE	Gallic acid equivalents
HMF	Hydroxymethylfurfural
LOD	Limit of detection
LOQ	Limit of quantification
RID	Differential refractive index detector
TBARS	Thiobarbituric acid reactive substances
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TFC	Total flavonoid content
TPC	Total phenolic content

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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 these feed on plant sap. The honeydew honey 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 [1, 2]. Several authors have reported the production of honeydew by insects in different European Coniferae such as *Abies*, *Picea*, *Larix* and *Pinus* [3, 4], and also in different *Quercus* species [5–7]. In Spain, the main sources of honeydew are holm-oak (*Quercus ilex*) and pyrenean oak (*Q. pyrenaica*), the latter especially in the Northwest of the country [8, 9].

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 liberated sweet sap contains natural sugars and minerals, and is ingested by bees and deposited in hives as a dark honey [6, 10].

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 have been indicated in various studies [9, 11–13]. These studies showed 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, since honeydew honeys were generally characterised as darker than nectar honeys [14]. However, it has been reported no significant differences for moisture and water activity between honeydew and nectar honeys [11]. 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 [15]. 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 [11, 16].

Honey is known for being part of traditional medicine due to its therapeutic properties. These properties are related to antioxidant activity of honey, being phenolic compounds, mainly flavonoids, and minerals, which are 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 showed higher antioxidant activities than nectar honeys and this may be related to the higher values of phenolic compounds in honeydew honey [9, 14–17].

Honeydew honeys are widely produced and consumed in Spain due to their functional importance. However, studies on the characterisation of this type of honeys are still scarce. In this sense, the studies of palynological and physicochemical characterisation of the Galician (Northwest Spain) *Quercus pyrenaica* honeydew honeys and the geographical and palynological characterisation of Spanish oak honeydew honeys can be highlighted [9, 18, 19]. Traditionally, a honeydew honey element/number of pollen "HDE/Pn ratio" > 3 was expected to be a characteristic of honeydew honeys. However, the last palynological characterisation of honeydew honeys in different countries [18, 19] showed very low values of this ratio, and suggested that this traditional ratio should not be considered as an indicator of honeydew honeys.

The aim of this work was to characterise a wide amount of Spanish oak honeydew honeys with two purposes: at first, to evaluate the physicochemical characteristics [moisture, pH, acidity, electrical conductivity, ash, and hydroxymethylfurfural (HMF) content] and colour parameters to verify that honey samples are certainly honeydew honeys; Second, considering the growing interest in honeydew honeys due to their nutritional and bioactive properties, the chemical composition (mineral elements, sugars, and total contents of phenols and flavonoids) and antioxidant activity (ability to scavenge radicals and lipid peroxidation inhibition) of these honeys were determined.

Materials and methods

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. In addition, different physicochemical parameters were evaluated to certify samples as honeydew honeys (Section Physicochemical parameters).

Reagents and standards

Formic acid, hydrochloric acid, trichloroacetic acid, HPLC-grade acetonitrile, methanol, ethanol, sodium carbonate, potassium persulfate, and Folin–Ciocalteu reagent were obtained from Panreac (Barcelona, Spain). 2-Thiobarbituric acid was obtained from Merck (Madrid, Spain). 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), sodium nitrite, aluminum trichloride, and sodium hydroxide were purchased from Fluka (Madrid, Spain). Hydrogen peroxide and nitric acid were obtained from Merck (Darmstadt, Germany). Tris(hidroxymethyl)amino-methane, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), phosphatebuffered saline (PBS), cumene hydroperoxide, and standards of gallic acid, catechin, HMF, fructose, glucose, sucrose, turanose, maltose, trehalose, isomaltose, melezitose, and raffinose were purchased from Sigma-Aldrich (Madrid, Spain). All dilutions were prepared with deionised

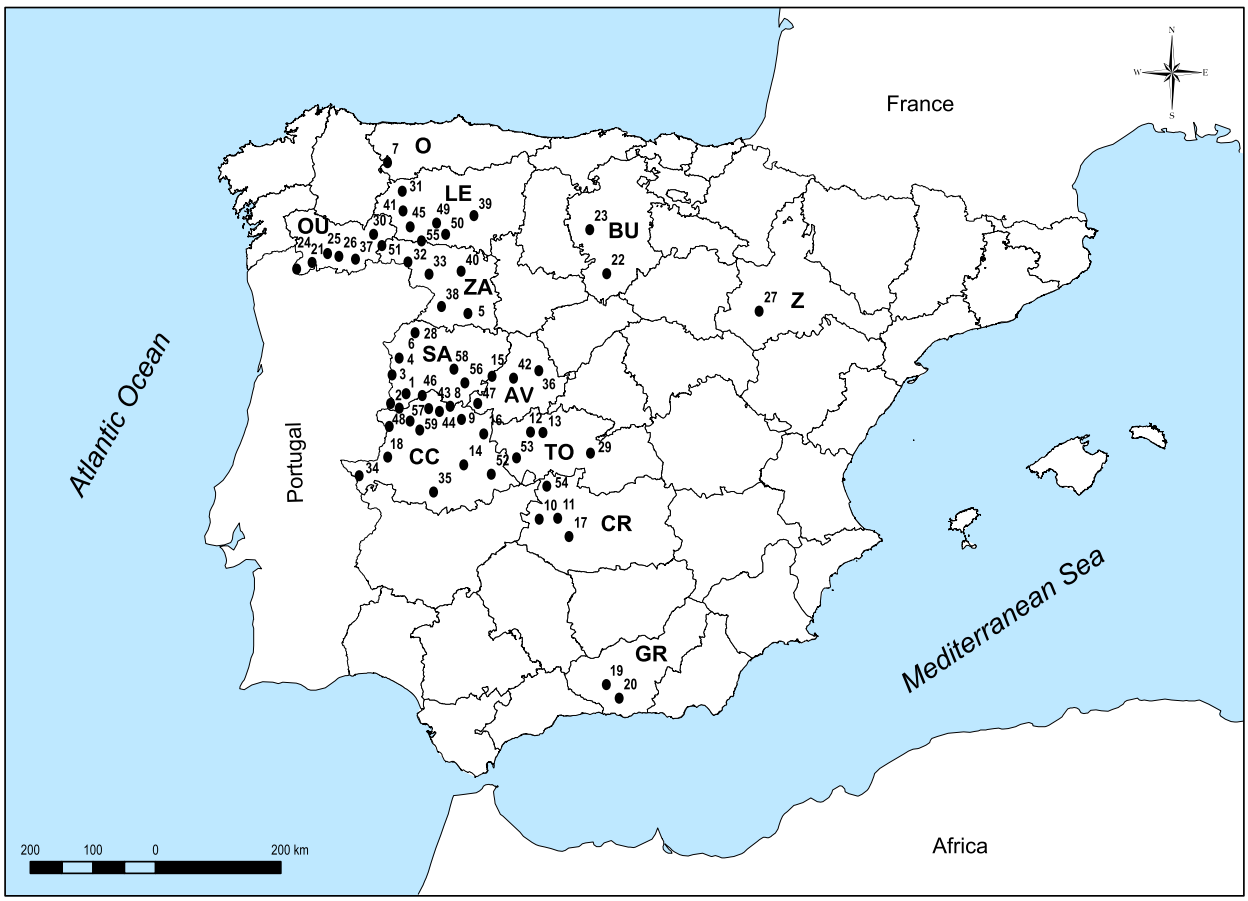

Fig. 1 Distribution of the honeydew honey samples studied by provinces. *AV* Ávila, *BU* Burgos, *CC* Cáceres, *CR* Ciudad Real, *GR* Granada, *LE* León, *O* Oviedo; *OU* Orense, *SA* Salamanca, *TO* Toledo, *ZA* Zamora, *Z* Zaragoza

water produced by a Milli-Q water purification system (Millipore, Belford, USA).

Physicochemical parameters

Physicochemical parameters such as moisture, pH, free, lactonic and total acidity, electrical conductivity, and ash were determined according to the official methods of analysis [20].

The identification and quantification of HMF was by Ultra Rapid Resolution Liquid Chromatography equipped with a diode-array detector (UHPLC-DAD) following the method described by Jara-Palacios et al. [21]. Honey samples were accurately weighed (5 g) and 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 standards. Results were expressed as mg of HMF per 1 kg of honey (mg/kg).

Colour parameters

Colour was assessed by tristimulus from the colourimetry based on reflectance spectra. The spectra were 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 [22]. The following CIELAB parameters were assessed: L^* (lightness), a* and b* (two colour coordinates), h_{ab} (hue angle), and C^*_{ab} (chroma).

Chemical composition

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. Samples were prepared from exactly 0.4 g put into polytetrafluoroethylene vessels, and 7 mL of HNO_3 and 1 mL of H_2O_2 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 °C, 10 min 850 W at 170 °C, 5 min 850 W at 200 °C, 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 acid used for the digestion was prepared in the same way. Fourteen minerals (Al, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, Pb, S, Si, and Zn) were determined in each honey and results were expressed as mg of mineral per 1 kg of honey (mg/kg).

Sugar profile

The development and validation of an HPLC method was carried out to determine the sugar composition of honeydew honeys.

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 using a ZORBAX Carbohydrate Analysis column (4.6 × 250 mm) with a particle-size diameter of 5 µm. The column was maintained at 30 °C throughout the analysis. The mobile phase was composed of 75% acetonitrile in water. The injection volume was 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 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. 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. Results were expressed as mg of sugar per 100 g of honey (mg/100 g).

The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the calibration curves [23]. 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 °C.

Approximately 1 g of honey was weighed and mixed with 10 mL of 15% acetonitrile. 1 mL of the dissolution was then filtered through a hydrophilic PVDF Millex-HV 0.45 µm syringe filter prior to HPLC analysis. All the samples and standards were injected twice to obtain the averages.

The developed method allowed 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 were of 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 correspond 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.

Total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu assay with some modifications as reported previously [24]. Gallic acid was employed as a calibration standard and results were expressed as mg gallic acid equivalents per 100 g of honey (mg GAE/ 100 g). Three replicates from each sample were analysed.

Total flavonoid content

Total flavonoid content (TFC) was determined by the aluminum chloride colorimetric method as previously described [25]. Honey sample (500 µL) 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 equivalents per 100 g of honey (mg CE/100 g). Three replicates from each sample were analysed.

Antioxidant activity

ABTS/persulfate assay

The ability to scavenge the $ABTS^{\bullet+}$ radical was measured in vitro based on the ABTS assay [26]. Honey sample (50

µL) was added to 2 mL of the $ABTS^{\bullet+}$ diluted solution (7 mM ABTS with 2.45 mM potassium persulfate) and the absorbance was measured at 734 nm after 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). Three replicates from each sample were analysed.

Thiobarbituric acid reactive substances (TBARS) assay

The lipid peroxidation inhibition was determined by the TBARS assay [27], with some modifications [28]. Livers of rats were homogenised in 20 mM Tris-HCl buffer (pH 7.5) and centrifuged for 10 min at 3000*g*. The supernatant (200 µL) was mixed with 100 µL of honey sample, 25 µL of 20 mM cumene hydroperoxide (oxidant compound), and Tris-HCl buffer up to a total volume of 1 mL. The mixture was incubated at 37 °C for 1 h, and then, 10% trichloroacetic acid at 4 °C was added and the mixture was centrifuged at 3000*g* for 10 min. Finally, 1 mL of 2-thiobarbituric acid was added to the supernatant and 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). Three replicates from each sample were analysed.

Statistical analysis

Correlations by both simple and multiple regressions computed by general linear models (GLM) were studied between: (a) chemical composition (TPC, TFC, and mineral content) and antioxidant activity (measured by ABTS and TBARS assays); (b) mineral content and colour parameters. The Statistica© v.8.0 software was used for all the statistical treatments [29].

Results and discussion

Physicochemical parameters

The results of physicochemical parameters (moisture, pH, acidity, electrical conductivity, ash, and HMF) of honeydew honeys 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 [30]. Similar low levels of water content in Polish honeydew honeys were found by Rybak-Chmielewska et al. [7]. Persano Oddo and Piro [5] also determined the average water content at 16% in European honeydew honeys.

Table 1 Physicochemical parameters in Spanish *Quercus* honeydew honeys

	Mean	SD	Min	Max
Moisture (%)	16.5	1.5	13.4	20.0
pH	4.74	0.18	4.34	5.14
Free acidity (meq/kg)	39.5	4.3	30.9	52.1
Lactonic acidity (meq/kg)	3.43	1.42	0.92	8.72
Total acidity (meq/kg)	43.0	5.1	33.6	56.8
HMF (mg/kg)	3.33	2.00	1.32	13.41
Electrical conductivity (µS/cm)	1009	132	811	1363
Ash (%)	0.68	0.13	0.38	1.13

SD standard deviation, *Max* maximum value, *Min* minimum value

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) [7, 9]. In addition, Croatian and Macedonian honeydew honeys showed very similar values (mean = 4.8 and 4.7, respectively) [31].

The values for the free acidity ranged between 30.9 and 52.1 meq/kg. According to the EU legislation [30], the upper limit for free acidity is 50 meq/kg and only honey sample 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, [31]), and relatively higher than those found in pine Greek honeys (range 23.75–44.94 meq/kg, [32]).

According to EU legislation, the lower limit value of electrical conductivity for honeydew honey is 800 µS/cm. The present 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*) [5, 7, 9, 32, 33].

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 Greek pine and fir honeys [32], but much higher than those found in honeydew honeys from the Soria Province (Spain) [34].

Regarding HMF, 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.

Colour parameters

Table 2 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 CIELAB units. As all samples showed $L^* < 55$ CIELAB units, these can be classified as dark honeys. The chroma (C^*_{ab}) values ranged between 11.87 and 41.37 CIELAB units (mean = 31.89 units), and the hue (h_{ab}) ranged between 42.92° and 73.80°. Figure 2 shows the projection of the colour points corresponding to each honey sample on the a*b*-colour diagram. In the a*b*-colour diagram, it can be observed that honeydew honeys are classified into two different groups according to the hue: a first group of 50 samples with h_{ab} values > 55°, in the yellowish-orange zone, and a second group of nine samples with h_{ab} values from 40° to 55°, in the orange-red zone. The second group showed the lowest L^* values (< 34.43 CIELAB units), indicating darker colours than the first group. These values found for L^*, C^*_{ab}, and h_{ab} are in agreement with the results obtained by Gonzalez-Miret et al. [35] for honeydew honeys.

Determination of minerals

The contents of the minerals quantified in honeydew honey samples are shown in Table 3. The average total mineral content was 2500 mg/kg. The most abundant element in the honeys analysed was K, which has an average content of 1845 mg/kg, and accounted for 73% of the total minerals quantified; this finding coincides with those of the majority of authors in the literature, who reported this mineral to be the most abundant in honey. Italian and Polish honeydew honeys have shown a mean content of K of 2569 and 2387 mg/kg, respectively [36, 37].

The second and third most abundant minerals are P and Mg, with average values of 211 (8.46%) and 188 mg/kg (7.54%), respectively, while Ca (mean = 106 mg/kg) and S (mean = 87 mg/kg) accounted for 4.25 and 3.5%, of the total minerals quantified, respectively. Other minerals (Al, Cu, Fe, Ni, Mn, Na, Pb, Si, and Zn) accounted for less than 1% of the total minerals quantified. Several minerals, such as Fe and Na, were 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 Mg and Mn were present in greater quantities with respect to the Greek, Polish, Italian, and Anatolian pine honeys [36–41].

Table 2 Colour variables measured by diffuse reflectance method in the CIE 1976-L^* a* b* (CIELAB) colour space in Spanish *Quercus* honeydew honeys

	Mean	SD	Min	Max
L^*	41.08	8.12	19.59	54.57
a*	12.88	3.71	7.82	30.29
b*	28.89	6.42	8.39	37.51
C^*_{ab}	31.89	6.16	11.87	41.37
h_{ab}	65.15	7.99	42.92	73.80

SD standard deviation, *Max* maximum value, *Min* minimum value

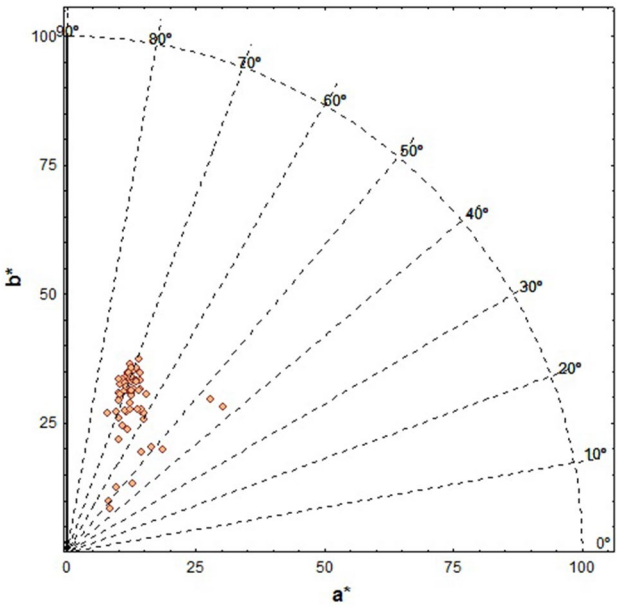

Fig. 2 Distribution of the Spanish *Quercus* honeydew honeys within CIELAB colour space (a*b*-diagram)

Table 3 Mineral content (mg/kg) in Spanish *Quercus* honeydew honeys

	Mean	SD	Min	Max	%[a]
Al	0.85	1.13	0.08	4.89	0.03
Ca	106.0	52.3	25.1	282.0	4.25
Cu	1.23	0.39	0.61	3.14	0.05
Fe	1.91	2.03	0.04	12.00	0.08
K	1845	267	1390	2428	73.98
Li	0.37	0.20	0.23	1.01	0.01
Mg	188.0	60.5	64.0	327.0	7.54
Mn	22.5	14.4	0.3	61.0	0.90
Na	20.9	8.0	8.4	39.5	0.84
P	211	31	105	266	8.46
S	87.3	19.1	41.3	129.0	3.50
Si	7.96	4.01	3.14	27.10	0.32

SD standard deviation, *Max* maximum value, *Min* minimum value

[a]Percentage content of each mineral of the total mineral quantified

Determination of sugars

The sugar quantification is summarised in Table 4. The total content of sugars ranged from 63.42 to 73.43 g/100 g, with a mean value 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 value of 57.29 g/100 g. The total content of monosaccharides was generally lower than those found in Greek (mean *Pinus* = 77.90 g/100 g; mean *Abies cephalonica* = 75.15 g/100 g), Macedonian (mean = 70.4 g/100 g), Croatian (mean = 63.3 g/100 g), Polish (mean *Abies alba* = 62.00 g/100 g), and Turkish honeydew honeys (mean *Quercus* = 65.01 g/100 g, mean *Pinus* = 63.47 g/100 g) [7, 16, 31, 33].

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 4–11 g/100 g lower than the content of fructose. The fructose content lay between 30.05 and 37.98 g/100 g, with a mean of 32.58 g/100 g. The results of fructose differed only slightly from those of other European countries [5], with an average of 32.5 g/100 g, ranging from 28.7 to 36.2 g/100 g, while the present 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). The fructose/glucose ratio (*F*/*G*) ranged from 1.14 to 1.55, with a mean of 1.32. With respect to the F/G ratio, present results agree with those found: by Persano Oddo and Piro [5] (mean *F*/*G* = 1.25); by Rybak-Chmielewska et al. [7] (mean *F*/*G* = 1.2); and by Golob and Plestenjak [42] (mean *F*/*G* = 1.35), in European, Polish, and Slovenian honeydew honeys, respectively.

Table 4 Sugar content (g/100 g) in Spanish *Quercus* honeydew honeys

	Mean	SD	Min	Max
Monosaccharide				
Fructose	32.58	1.48	30.05	37.98
Glucose	24.71	1.29	19.67	27.07
Disaccharide				
Sucrose	0.17	0.20	0.01	1.31
Turanose	2.60	0.27	1.97	3.25
Maltose	4.90	0.99	2.23	6.95
Trehalose	2.03	0.64	0.76	3.65
Isomaltose	1.46	0.48	0.62	3.19
Trisaccharide				
Melezitose	0.64	0.33	0.32	1.49
Raffinose	0.04	0.02	0.01	0.10
F+G	57.29	2.30	50.19	64.46
F/G	1.32	0.08	1.14	1.55
ΣSugars[a]	69.16	2.12	63.42	73.43

F + G: sum of fructose and glucose. F/G: fructose/glucose ratio

SD standard deviation, *Max* maximum value, *Min* minimum value

[a]Sum of all sugars quantified in each honey sample. F: fructose. G: glucose

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 was 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. It was noticeable that disaccharides were 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 et al. [43] and Persano Oddo and Piro [5], who reported an average value of 0.98, 0.2, and 0.8 g/100 g, respectively. The present results concerning maltose content are in agreement with those of the literature, which ranged from 1.9 to 4.4% in fir honeydew honey (*Abies alba*) [7], and from 3.43 to 6.22% in Macedonian honeydew honey [31]. The results of those remaining disaccharides quantified in the present study showed, in general, similar values to those of Polish fir honeys, whereas turanose and trehalose showed a mean content of 1.8 and 2.7 g/100 g, respectively [7].

Regarding the trisaccharide content, the melezitose was quantitatively the most important, ranging from 0.32 to 1.49 g/100 g with a mean value of 0.64 g/100 g, while raffinose presented a low mean value of 0.04 g/100 g.

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 [7, 33]. In general, the presence of melezitose in the samples confirms that a substantial part of the analyzed honeys was of honeydew.

Determination of phenolic and flavonoid content

Table 5 summarises the TPC (mg GAE/100 g) and TFC (mg CE/100 g) contents determined 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, the present results are consistent with those found in Romanian (mean = 127 mg

Table 5 Total phenolic content (mg GAE/100 g), total flavonoid content (mg CE/100 g), and antioxidant activity (ABTS and TBARS assays: µmol TE/100 g and % inhibition, respectively) in Spanish *Quercus* honeydew honeys

	Mean	SD	Min	Max
TPC	130.25	49.26	50.04	243.86
TFC	11.30	4.46	1.81	25.22
ABTS	858.77	353.23	234.65	2252.78
TBARS	27.55	11.43	10.49	53.38

SD standard deviation, *Max* maximum value, *Min* minimum value

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 [9, 33, 44].

Antioxidant activity

The antioxidant activity values measured by the ABTS assay ranged from 234.64 to 2252.78 µmol TE/100 g (Table 5). The obtained results indicate that honeydew honeys have a high antioxidant activity, which could be related to the high phenolic content [15, 17]. A previous study indicated that the oak honeys had higher total phenolic content and higher antioxidant capacity measured by ABTS method than blossom honeys [40]. According to the literature, phenolic compounds are one of the most important antioxidant compounds found in honey [45], and the flavonoid content is highly related to the antioxidant activity [14, 15]; however, this relationship was not confirmed in the present 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. The 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 an in vitro biological system (Table 5). Ferreira et al. [46] studied the capacity of three varieties of honeys (light, amber, and dark) to inhibit the lipid peroxidation in brain tissue from pigs, and concluded that dark honey showed, in all the assays, a better antioxidant activity (lower EC50 values) than the other honey samples (amber and light). As far we know, no previous studies regarding inhibition of lipid peroxidation of honeydew honeys have been published.

Correlation among the investigated parameters

Correlations analyses were applied to explore relationship between the contents of phenolics, flavonoids, and minerals, and the results of antioxidant activity. Significant and low linear correlations were found between TBARS values or ABTS values and the contents of phenolics, flavonoids, and minerals ($p < 0.05$; $R = 0.27$ 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 [9, 47, 48]. 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.

In addition, correlations between the minerals and colour parameters were determined. Results indicated that lightness was significantly correlated with some individual minerals: Cu, Fe, K, Mg, Mn, and S, although a low multiple correlation coefficient was found ($r^2 = 0.34$). In a previous study from our group [35], the darkest honey 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, Fe, As, Pb, and Cd.

Conclusion

In this study, the 59 honey samples from different regions of Spain were characterised as honeydew honeys, because 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 were present in greater quantities with respect to the other honeydew honeys from other regions, while total content of monosaccharides was much lower. In general, 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 these honeys does not seem to be a property of a single phytochemical compound, but it is correlated both to phenolic compounds and minerals. This study represent a contribution to the characterization of honeydew honeys and 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.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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