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Phenolic compounds and color of labeled resin spurge honey and their correlations with pollen content

Dolores Hernanz [a], M. Ángeles Palomar [a], Abdelkarim Moujanni [b], Abdelkhalid Essamadi [b], Francisco J. Heredia [c], Anass Terrab [d,*]

[a] Department of Analytical Chemistry, Universidad de Sevilla, Facultad de Farmacia, 41012, Sevilla, Spain
[b] Laboratory of Biochemistry & Neuroscience, Applied Biochemistry and Toxicology Team, Hassan First University, Faculty of Sciences and Technology, BP577, Settat, 26000, Morocco
[c] Food Colour & Quality Lab. Facultad de Farmacia, Universidad de Sevilla, 41012, Sevilla, Spain
[d] Departamento Biología Vegetal y Ecología, Universidad de Sevilla, Apdo. 1098, 41080, Sevilla, Spain

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ABSTRACT

Our aim in this study was to outline phenolic and color characteristic that characterize the labeled unifloral resin spurge (*Euphorbia resinifera*) honey. With respect to phenolic composition, 17 phenolic compounds have been analyzed in the 29 honey samples. The proposed markers (syringic acid, ethyl gallate, *m*-coumaric acid and naringenin) might help to the enhancement of this honey type and thus, guarantee its commercial value. The color characterization by diffuse reflectance spectrophotometry revealed typical values of light amber honey (lightness ranged from 36 to 70 units, and chroma from 18 to 30 units). On the other hand, many correlations between the color attributes and phenolic acids, total phenolic compounds, caffeic acid, *p*- and *m*-coumaric acids and hesperidin have been demonstrated, also, correlation between phenols, color parameters and percentage of pollen of *E. resinifera* has been found. This study is one of the rare researches which have correlated the CIELAB color parameters with the individual phenolic acids and flavonoid compounds within the same unifloral honey.

1. Introduction

Traditionally, the pollen analysis has been used to identify the botanical origin of the honey. Nevertheless, many researchers suggest that physicochemical characteristics could be more exhaustive and precise in the characterization of the honey (Karabagias, Halatsi, Karabournioti, Kontakos, & Kontominas, 2017). In this way, the use of phenolic compound analysis in the identification and characterization of honeys has been suggested and has since been used as tool for studying the botanical and geographic origins of honeys. In addition, the quantitative analysis of phenolic compounds, comprising phenolic acids and flavonoids in honey is not only to assess their potential markers of botanical origin, but also for their contributions to overall bioactivity of honey (Pasupuleti, Sammugam, Ramesh, & Gan, 2017; The National Honey Board, 2003). On the other hand, and despite their importance, no official method with respect to phenols assessment on honey was suggested so far.

Different methods were carried out to determine phenolic compounds in honey (Ciulu, Spano, Pilo, & Sanna, 2016). The analysis of phenols in honeys is always complex, because to the large number of compounds present in slight quantities and to the complexity in the preparation and analysis of the sample. The combination of solid-phase extraction cartridges for sample preparation and high performance liquid chromatographic (HPLC) analyses has been successfully applied for the determination of phenolic compounds in honey (Pascual-Maté, Osés, Fernández-Muiño, & Sancho, 2018). In recent times, a patent advancement in chromatographic performance was obtained by the establishment of techniques as rapid-resolution liquid chromatography (RRLC) or ultra-performance liquid chromatography (UHPLC) (Campone et al., 2014; Gašić et al., 2015; Kečkeš et al., 2013; Stinco, Benítez-González, Hernanz, Vicario, & Meléndez-Martínez, 2014; Stinco, Benítez-González, Meléndez-Martínez, Hernanz, & Vicario, 2019; Trautvetter, Koelling-Speer, & Speer, 2009).

Many phenolic compounds have been considered as a biomarkers, e.g. methyl syringate for asphodel honey (Tuberoso et al., 2009), homogentisic acid for strawberry tree honey (Rosa et al., 2011), phenyllactic acid for thistle (*Galactites tomentosa*) honey, kaempherol for rosemary honey, and caffeic, *p*-coumaric and ferulic acids for chestnut honey

* Corresponding author.
E-mail address: anass.terrab@gmail.com (A. Terrab).

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Fig. 1. Image illustrating the honey production area of labeled resin spurge honey: *Euphorbia resinifera* (Morocco). (Photo: Mouj@nni®).

(Gómez-Caravaca, Gómez-Romero, Arráez-Román, Segura-Carretero, & Fernández-Gutiérrez, 2006; Tomás-Barberán, Ferreres, Blázquez, Garcia-Viguera, & Tomás-Lorente, 1993).

On the other hand, industrial processing methods, temperature and/or time of storage or relation of honey color to the botanical origin have been subject of several studies (Gonzales, Burin, & del Pilar Buera, 1999; Wilczyńska, 2014). Additionally, the influence that the pollen grains and mineral content have on honey color were also explored (González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005; Terrab, Escudero, González-Miret, & Heredia, 2004).

The genus *Euphorbia* contains irritant latex (called euphorbium) and a large number of them were used for medicinal purposes. Resin spurge (*Euphorbia resinifera* Berg; Fig. 1), is an endemic species of the Middle Atlas mountains (Morocco), like medicinal plant, it comprises a great contents of resiniferatoxin and lot of triterpenoids, which are used against pain treatment and cancer (Appendino & Szallasi, 1997; Fattorusso, Lanzotti, Taglialatela-Scafati, Tron, & Appendino, 2002; Leong & Copenhaver, 2018, pp. 861–868; Wang et al., 2016). Resin spurge honey is considered one of as among the most appreciated honey types (Ricciardelli D'Albore, 1998), because it's peppery taste and pungent aroma (Agricultural Ministry of Agriculture Ministry of Morocco, 2015), and cosmetic attributes (Benlyas, Alem, & Filali-Zegzouti., 2016; Khiati et al., 2013). *E. resinifera* honey is Protected Geographical Indication (PGI) produced in unique covered area (wild and spontaneous plant) over than 8.000 ha (Agricultural Ministry of Agriculture Ministry of Morocco, 2015).

Our aim is to provide suitable phenolic and chromatic markers for the authentication and characterization of the *E. resinifera* honey. Thus, (1) the development and validation of UHPLC method for the determination of phenolic compounds, and (2) the CIELAB color attributes were carried out in 29 unifloral resin spurge honey samples. Furthermore, many statistical approaches have been achieved to explore the correlation between the different phenolic compounds analyzed, color and pollen contents.

2. Materials and methods

2.1. Honey samples

29 samples of unifloral *E. resinifera* honey have been purchased in the PGI zone between 2013 and 2014. The uniflorality of the samples was previously achieved by the melissopalynological study (Terrab et al., 2022), which set the percentage of pollen of *E. resinifera* required in > 20% to be considered unifloral of resin spurge.

2.2. Phenolic compounds

2.2.1. Standards and reagents

The extraction and chromatographic solvents were of analytical and HPLC grade. Acetonitrile, methanol, and ethyl acetate were purchased from Merck, Darmstadt (Germany). Formic acid was procured from Fluka. Water was purified in a Nanopure®DiamondTM system (Barnsted Inc. Dubuque, IO). Gallic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, ethyl gallate, *p*-coumaric acid, *m*-coumaric acid, ferulic acid, hesperidin, naringenin, naringin, pinocembrin, quercetin, quercetrin, chrysin and galangin were obtained from Sigma Aldrich (Madrid, Spain).

2.2.2. Extraction procedure

Following the method described by Gheldof, Wang, and Engeseth (2002), the extraction process has been made but with some modifications. Honey (1 g) was dissolved in 3 mL of acidified water (pH 2) and filtered through a column of Amberlite XAD-2 SPE (300 mg; 20–60 mesh size, SUPELCO-Sigma-Aldrich, USA), which was previously conditioned with 3 mL of methanol, ultrapure water, and acidified water (pH 2). The column was washed with 3 mL of water and acidified water (pH 2) to remove sugar and polar compounds and phenolic compounds were eluted with 3 mL of methanol. Under reduced pressure this extract was concentrated to dryness. The residue obtained was dissolved in 500 μL of water and extracted with ethyl acetate (500 μL x 3). Under reduced pressure the organic extracts have been combined and concentrated. The residue has been first mixed in 200 μL of 0.01% formic acid, then filtrated through a hydrophilic PVDF Millex-HV 0.45 μm syringe filter

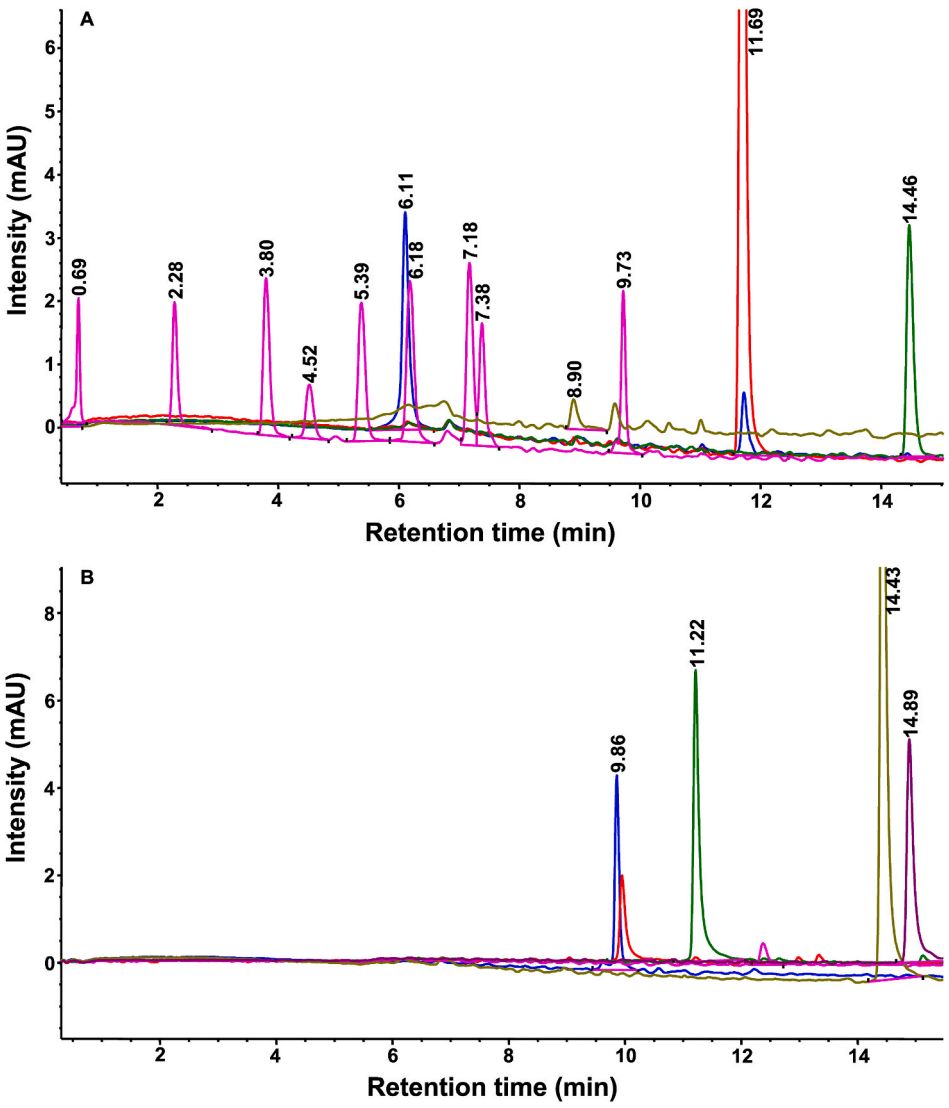

Fig. 2. Chromatograms of a mixture of standards at different wavelengths (A) 280 nm and (B) 320 nm. 1. gallic acid (tr: 0.69 min), 2. *p*-hydroxybenzoic acid (tr: 2.28 min), 3. vanillic acid (tr: 3.80 min), 4. caffeic acid (tr: 4.52 min), 5. syringic acid (tr: 5.39 min), 6. ethyl gallate (tr: 6.11 min), 7. *p*-coumaric acid (tr: 6.18 min), 8. *m*-coumaric acid (tr: 7.18 min), 9. ferulic acid (tr: 7.38 min), 10. hesperidin (tr: 8.90 min), 11. naringin (tr: 9.73 min), 12. naringenin (tr: 11.69 min), 13. pinocembrin (tr: 14.46 min), 14. quercetin (tr: 9.86 min), 15. quercetrin (tr: 11.22 min), 16. chrysin (tr: 14.43 min), 17. galangin (tr: 14.89 min).

(Millipore, Bedford, MA, USA), and lastly analyzed by UHPLC.

2.2.3. Ultra high performance liquid chromatography conditions (UHPLC)

Analyses were carried out in an Agilent Technologies UHPLC 1260 Infinity chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode-array detector, which was set to scan from 200 to 770 nm, and a C18 Eclipse plus column (1.8 μm, 5 cm × 2.1 mm) using an injection volume of 1 μL. The mobile phase (Solvent A) was 0.01% of formic acid in water and acetonitrile (solvent B) at the following gradient: 0–5 min, 5% B linear; 5–20 min 50% B linear; 20–21 min, washing and re-equilibration of the column. The temperature of the column was set at 20 °C and the flow rate was 1 mL/min. The identification has been realized by comparison of their chromatographic and UV/vis spectroscopic characteristics with those of standards.

2.2.4. Analytical parameters

The external standard calibration approach has been used, from the areas of the chromatographic peaks obtained by DAD detection at 280 nm at 370 nm, in order to quantify the 17 phenolic compounds. Linearity of the method has been cheeked considering the detector response to diverse concentrations of phenolic compounds using linear regression. The different phenolic standards have been prepared in acetonitrile at [100 mg/mL], and the calibration curves have been made up of six dilutions of the stock solutions in formic acid (0.01%). By the use of the Microcal Origin ver. 3.5 software (Origin Lab Corporation, Northampton, MA, USA), the limit of quantification (LOQ) and detection (LOD) have been estimated from the calibration curves. The LOQ have been assessed as ten times the relative standard deviation (RSD) of the analytical blank rates, whereas, the LOD have been estimated as three times the RSD.

The repeatability (precision) was evaluated by the relative standard deviation of six independent assays performed under the same analytical conditions in the shortest period of time (UNE 82009–1:1998).

A recovery analysis has been carried out to corroborate the validity of the technique. For this, a standard solution has been made up in acetonitrile at [30 mg/L] of phenolic acids and 50 mg/L of flavonoids. To check the recovery of the different phenols, the sample of honey was spiked with 20 μl of standards solution and then extracted following the method developed in section 2.2.2. Lastly, the samples were checked by means of the UHPLC method proposed. The recovery rate has been estimated by the ratio $(C_1/C_2) \times 100$, for each phenolic compound, where C_1 is the means of measured concentrations in sample, and C_2 is the amount of the analyte added to honey (UNE 82009 Standards). All honey samples have been extracted in duplicate, whereas, in order to get the averages, the standards and honey samples have been loaded three times.

Table 1
Phenolic acids, flavonoids and phenols contents of the 29 *Euphorbia resinifera* honey samples analyzed (mg/100 g).

	Mean	SD[a]	Min	Max
Gallic acid	0.075	0.041	0.019	0.204
p-Hydroxybenzoic acid	0.230	0.116	0.041	0.518
Vanillic acid	0.105	0.080	0.023	0.488
Syringic acid	1.101	2.514	0.087	13.389
Ethyl gallate	0.605	0.282	0.081	1.350
Caffeic acid	0.034	0.055	nd	0.288
p-Coumaric acid	0.273	0.149	0.089	0.836
m-Coumaric acid	1.856	2.015	0.412	10.415
Ferulic acid	0.005	0.013	nd	0.048
ΣPhenolic acids[b]	4.284	3.461	1.440	17.502
Naringenin	0.448	0.176	0.068	0.764
Naringin	0.010	0.026	nd	0.125
Hesperidin	0.019	0.049	nd	0.152
Pinocembrin	0.065	0.100	nd	0.400
Chrysin	0.010	0.028	nd	0.103
Galangin	0.010	0.027	nd	0.112
Quercetrin	0.027	0.094	nd	0.504
Quercetin	0.001	0.004	nd	0.021
ΣFlavonoids[b]	0.597	0.291	0.108	1.296
ΣPhenols[b]	4.881	3.640	1.806	18.798

[a] SD: Standard deviation. nd: not detected.
[b] Σ, Phenolic acids, ΣFlavonoids, ΣPhenols total: sum of all individual phenolic acids, flavonoids, and phenolic compounds, respectively.

2.3. Color parameters

The reflectance spectra were measured directly on the honey with a CAS-140B spectroradiometer equipped with a ISP 80–111 sphere integration (Instrument Systems, Munich, Germany) and a IS-Specwin 1.8 data collection station (Instrument System, Munich, Germany) set to calculate in the visible spectra. Using the CromaLab® software, the spectra were integrated (Heredia, Álvarez, González-Miret, & Ramírez, 2004). The CIELAB uniform color space has been considered, and the color attributes have been calculated, lightness (L^*) and the CIELAB color coordinates, a^* and b^*. The hue angle (h_{ab}) and the chroma (C^*_{ab}), two color parameters correlated to tone and saturation, respectively, have been also determined.

2.4. Statistical analysis

Statistica v.8.0 software has been used for the statistical treatment of the data. Relationships between CIELAB color attributes and phenolic content were studied by both simple and multiple regressions computed by GLM. Correlations between phenolic content, color and percentage of *Euphorbia resinifera* pollen by pattern recognition techniques, e.g., Linear Discriminant Analysis (LDA), have been performed. In that case, statistically significant level was considered at $p < 0.05$.

3. Results and discussion

3.1. Analysis of phenolic compounds

3.1.1. Analytical characteristic

Using a mixture of standards and a honey extract, various attempts have been achieved in chromatographic conditions in order to get appropriate separation of the different phenols in the extracts. Chromatograms in the optimized conditions can be shown in Fig. 2. 17 phenolic compounds can be separated with the developed method in 20-min run. Three groups can be differentiated: 1) benzoic acids (gallic, *p*-hydroxybenzoic, vanillic, syringic acids and ethyl gallate); (2) hydroxycinnamic acids (ferulic, caffeic and *p*- and *m*-coumaric), and (3) flavonoids (quercetin, quercetrin, chrysin, galangin, naringenin, naringin, hesperidin and pinocembrin).

The validation of the different parameters of each calibration curve can be seen in Table S1 (see Supplementary materials). High linearity ($r^2 > 0.99$) in the range of concentrations has been found in all the curves. Vanillic acid shows the lowest LOD and LOQ (0.07 ng and 0.23 ng, respectively), while naringin shows the highest ones (1.32 ng and 4.39 ng, respectively).

The mean recovery of the standards has been made to determine the validity of the method. The values of recovery ranged between 37 and 87% for caffeic and *p*-coumaric acids, respectively. The repeatability was evaluated by considering the relative standard deviation. RSD values ranged between 0.26 and 11.55% for pinocembrin and caffeic acid, respectively.

3.1.2. Analysis of phenolic compounds in Euphorbia resinifera honeys by UHPL method

17 phenols were recognized and quantified as phenolic acids i.e. (1) benzoic acids (gallic, *p*-hydroxybenzoic, vanillic, syringic acids and ethyl gallate); (2) hydroxycinnamic acids (caffeic, ferulic, *p*- and *m*-coumaric acids), and (3) flavonoids (quercetin, quercetrin, chrysin, galangin, naringenin, naringin, hesperidin and pinocembrin). Phenolic composition can be seen in Table 1 and shows that the predominant chemical compounds are hydroxycinnamic acids (43% of total phenolic compounds) followed by benzoic acids (42% of total phenolic compounds). Flavonoids are present in lower concentration (15% of total phenolic compounds).

In general, all of the 29 honey samples showed a common phenolic profile characterized by acids like gallic (0.019–0.204 mg/100g), *p*-hydroxybenzoic (0.041–0.518 mg/100g), vanillic (0.023–0.488 mg/100g), syringic (0.087–13.389 mg/100g), ethyl gallate (0.081–1.350 mg/100g), caffeic (nd-0.288 mg/100g), *p*-coumaric (0.089–0.836 mg/100g), *m*-coumaric (0.412–10.415 mg/100g) and ferulic (nd-0.048 mg/100g). The most abundant acids in the 29 samples were three, syringic (mean = 1.101 mg/100g), ethyl gallate (mean = 0.605 mg/100g) and *m*-coumaric (mean = 1.856 mg/100g), being *p*-hydroxybenzoic and *p*-coumaric present in intermediate contents (mean = 0.203 and 0.273 mg/100g, respectively). Other acids present in all the 29 honeys samples but in low quantities are gallic and vanillic (mean = 0.075 and 0.105 mg/100g, respectively). Regarding the flavonoids, it should be noted the high concentration of the naringenin in the samples (mean = 0.448 mg/100g), whereas the other seven flavonoid compounds detected were presents in few honey samples, with mean contents ranging from 0.065 mg/100g for pinocembrin to 0.001 mg/100g for quercetin (for more details see Table S2).

Studies done on phenolic compounds in *Euphorbia* honey are very scare, but Boutoub et al. (2021) identified 10 phenols in six samples of *Euphorbia* honey and detected abscisic, *p*-hydroxybenzoic, *p*-coumaric and gallic acids, and naringenin and quercetin in the two *E. resinifera* honey samples analyzed, but without given any concentration of them. Also, Kıvrak and Kıvrak (2017) evaluated 32 phenolic compounds in nineteen different honey types from Turkey, being euphorbia honey (without specifying the species) one of the honey types with the highest phenolic content (50.16 mg/100g).

Researchers conducted on unifloral honeys around the Mediterranean have found phenolic compounds that characterizing many honey types. E.g., *Erica* honeys from Portugal are recognized by the presence of syringic, *o*-coumaric, *p*-hydroxybenzoic and ellagic acids, whereas, *Lavandula* honeys by high concentrations of gallic acid (Andrade, Ferreres, & Amaral, 1997). Perna, Intaglietta, Simonetti, and Gambacorta (2013) have found in italian unifloral honeys high concentrations for gallic acid in eucalyptus and sulla honeys (mean = 5.3 and 4.01 mg/100g, respectively), and high concentration for ferulic acid and gallocatechin in chustnut honey (mean = 1.66 and 6.61 mg/100g, respectively). In Serbian unifloral honey high contents of gallic acid has been found in sunflower honey, and in buckwheat honey a greater content of apigenin has been found (Kečkeš et al., 2013). Whereas, in Greeck unifloral honeys Karabagias et al. (2014) found high amount of myricetin in honeydew and thyme honeys, and syringic acid in thyme

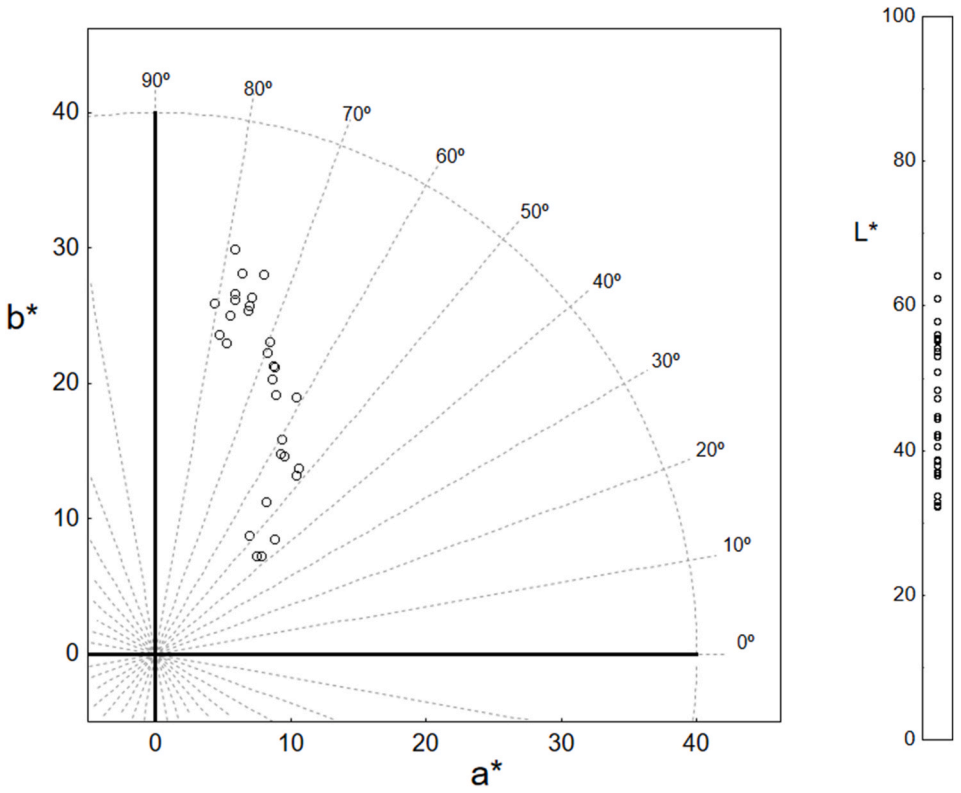

Fig. 3. Distribution of the *Euphorbia resinifera* honey samples within the CIELAB color space (a* b* -diagram) and lightness (*L**).

honey (mean = 4.27 mg/100g). As well, Tomás-Barberán, Martos, Ferreres, Radovic, and Anklam (2001) found high amounts of quercetin in sunflower honey, hesperitin in citrus honey and kaempferol in rosemary honey, and confirming the result of previous works in which these compounds as markers of these honey types.

The relative high amounts of syringic acid, ethyl gallate, *m*-coumaric acid and naringenin all together are uncommon in any type of honey. Thus, syringic acid have been found in some unifloral honey almost always in low amounts, e.g. in acacia, honeydew and buckwhert honeys (mean < 0.07 mg/100g, Biesaga & Pyrzyńska, 2013; Dimitrova, Gevrenova, & Anklam, 2007), in Australian manuka and kanuka honeys (mean = 0.134 and 0.135 mg/100g, respectively, Stephens et al., 2010), or in Yemeni jujube honey (mean = 0.172 mg/100g, Wabaidur et al., 2015), although, Michalkiewicz, Biesaga, and Pyrzynska (2008) reported syringic acid value of 1.80 mg/100g for heather honey. The highest values were detected in Chinese jujube and longan and in Turkish euphorbia honeys (mean = 4.250, 8.910 and 34.47 mg/100g, respectively; Kıvrak & Kıvrak, 2017; Zhao et al., 2016).

Regarding the *m*-coumaric acid and naringenin, all studies have found low to very low amounts for these compounds, in acacia and chestnut the mean *m*-coumaric acid varied from 0.141 to 0.202 mg/100g, respectively (Dimitrova et al., 2007), and in lavender and heather honeys the mean ranged from 0.06 to 0.11 mg/100g (Andrade et al., 1997), whereas the naringenin were found in very few unifloral honey and always with low quantities <0.05 mg/100g (e.g. acacia, honeydew, buckwheat and jujube honeys; Biesaga & Pyrzyńska, 2013; Wabaidur et al., 2015), while Campillo, Viñas, Férez-Melgarejo, and Hernández-Córdoba (2015) quantified this flavonoid in one sample purchased as rosemary (0.49 mg/100g), but without a melissopalynological confirmation. Finally, the ethyl gallate with a potent action against *Streptococcus mutans* (Gabe et al., 2019) has almost never been detected in honeys, while Bayram et al. (2020) quantified the ethyl gallate in various honey samples from different regions of Turkey but in very few concentrations.

3.2. Color parameters

The color of honey is very wide and can be ranged from white to a dark red (Shafiee, Minaei, Moghaddam-Charkari, Ghasemi-Varnamkhasti, & Barzegar, 2013). The results of the CIELAB color attributes can be seen in Table S3. The lightness (*L**) presented values ranging from 32 to 64 units (mean = 46.2 units), the chroma (C^*_{ab}) from 10 to 30 units and hue (h_{ab}), the qualitative attribute of color, between 43 and 80° (mean = 65.9°). Fig. 3 illustrates the location of each honey sample on the (*a**, *b**)- diagram and lightness. The samples were situated in a wide range of chroma and hue angles values. The CIELAB color characteristics of *E. resinifera* honeys show generally low values of chroma (mean C^*_{ab} = 21.6 units), *a** (mean = 7.7) and *b** (mean = 19.9). When comparing our color parameters results with those of honeys with similar variety like *Euphorbia officinarum* or *E. regis-jubae* (Bettar et al., 2019), we can conclude that parameters as chroma (mean = 44.4 and 74.8 units for *E. officinarum* and *E. regis-jubae*, respectively) or *b** (mean = 32.9 and 72.2 units for *E. officinarum* and *E. regis-jubae*, respectively), could perfectly differentiate our honey type from that of similar botanical origin. Other studies done in color of light (e.g. acacia, linden or rape), or amber-to-dark (e.g. eucalyptus, thyme or avocado) unifloral honeys using CIELAB color space, have found values of color attributes as *C**, *a** or *b** which could be distinguish these honey types from our resin spurge honey (Scripcă, Norocel, & Amariei, 2019; Terrab, Díez, & Heredia, 2003; Terrab, González-Miret, & Heredia, 2004).

3.3. Statistical analysis

3.3.1. Phenols vs color

Simple correlations have been applied to found relationships between the different CIELAB color attributes and the phenolic compounds (Table S4). Correlations ($p < 0.05$) between some color parameters (*L**, *b**, C^*_{ab}, h_{ab}) and the phenolic acids (r = −0.385, −0.402, −0.404 and −0.402, respectively), and total phenolic compounds (r = −0.371, −0.390, −0.394 and −0.388, respectively) have been found.

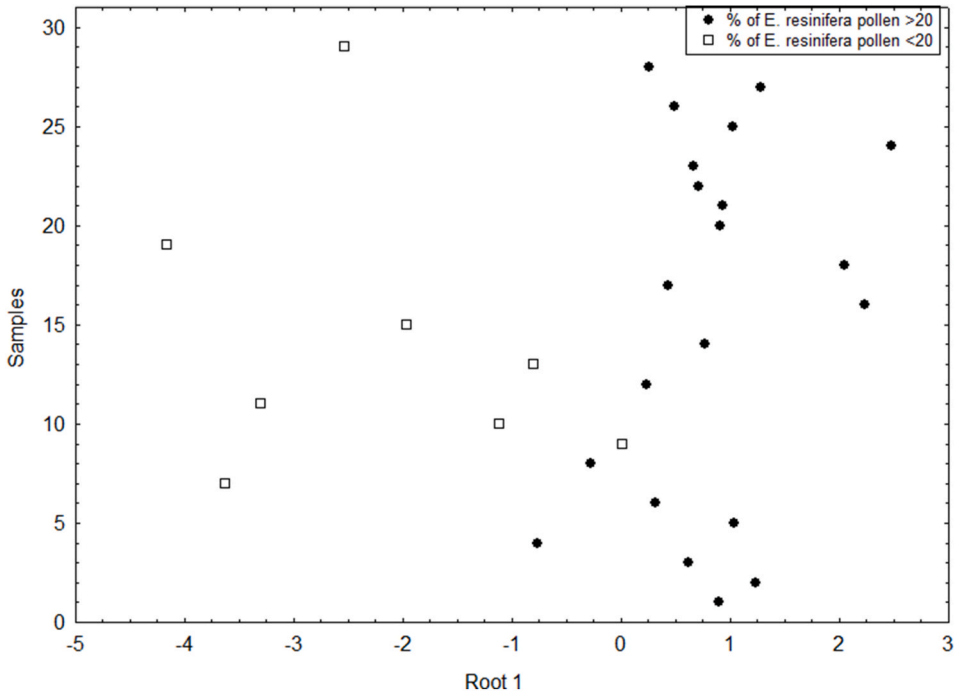

Fig. 4. Linear Discriminant Analysis (LDA) of the two group of honey samples according to their percentage of *E. resinifera* pollen as shown by a scatter diagram representing the projections of the points of each honey sample on the plane formed by the sole canonical variable.

Considering the individual phenol compounds significant relationships ($p < 0.05$) have been detected between b^*, C^*_{ab} and caffeic and *p*-coumaric acids, and between L^*, b^*, C^*_{ab}, h_{ab} and *m*-coumaric acid ($r = -0.400$, -0.377, -0.371 and -0.394, respectively). With respect to the flavonoid compounds, significant correlations have been detected only between hesperidin content and L^*, a^*, b^*, C^*_{ab}, and h_{ab} ($r = -0.409$, -0.386, -0.410, -0.404 and -0.396, respectively).

Research on honeys that have correlated color to phenols are frequent, the majority of these correlations have been made applying the Pfund method for color measurements and total phenolic and flavonoid contents, and very scare researches linked the CIELAB color parameters with the individual phenol and flavonoid compounds (Becerril-Sánchez, Quintero-Salazar, Dublán-García, & Escalona-Buendía, 2021) between unifloral honey, and non within the same unifloral honey. Thus, in Algerian honey a high correlation between phenolic compounds and color ($R^2 = 0.693$) has been found (Rebiai & Lanez, 2014). Highest phenolic and flavonoid values have been found in dark amber honeys (Al-Farsi, Al-Amri, Al-Hadhrami, & Al-Belushi, 2018). Also, a high positive relationship between color and both phenolic contents and flavonoid, has been found in honeys from different origins (Smetanska, Alharthi, & Selim, 2021). In the same way, Ferreira, Aires, Barreira, and Estevinho (2009) found a correlation between the color intensity and phenolic contents in honey samples from Portugal, and Moniruzzaman, Sulaiman, Khalil, and Gan (2013) detected a strong correlation between the color intensity of some Malaysian unifloral honeys and both phenolic and flavonoid contents. Daci-Ajvazi, Mehmeti, Zeneli, and Daci (2017) found that dark brown honeys from Kosovo show a larger phenol concentrations with respect to the light yellow ones, and exhibiting high correlation ($r = 0.711$) between phenol content and color. Similarly, Beretta, Granata, Ferrero, Orioli, and Facino (2005) noticed significant correlation between phenol content and color intensity in 14 commercial unifloral honey samples. In honeys from Mexico, a high correlation between color and flavonoid content values has been found (Balcázar-Cruz et al., 2019), showing a great correlation between a darker honeys and flavonoid content, which was also concluded in Argentinean honey from Chaco region (Cabrera, Perez, Gallez, Andrada, & Balbarrey, 2017), and in Brazilian multifloral honeys (Pontis, Costa, Silva, & Flach, 2014). In different Polish unifloral honeys, relationship between *p*-hydroxybenzoic acid and color intensity has been detected (Puścion-Jakubik, Karpińska, Moskwa, & Socha, 2022). Lastly, correlations made between CIELAB color parameters and total phenol and flavonoid contents on five Galician unifloral honeys (NW Spain) shown significant negative correlation between L^*, b^*, C^* and hue and polyphenol and flavonoid content, however, positive correlation have been found between polyphenol and flavonoid content and a^* (Escuredo, Rodríguez-Flores, Rojo-Martínez, & Seijo, 2019).

3.3.2. *Phenols and color vs pollen content*

As we mentioned, Terrab et al. (2022) established >20% of pollen of *E. resinifera* to consider an uniflorality of this honey variety, thus 21 samples had percentage > at 20% and eight samples lower than 20%. To establish differences between these two groups of samples with respect to their color and phenolic compounds, ANOVA and LDA were performed to evaluate the influence of the percentage of pollen from *E. resinifera* present in the 29 honey samples analyzed. High significance differences ($p < 0.05$) were found in the honey samples according to the percentage of pollen from *E. resinifera*, with benzoic acids ($p < 0.002$; $R^2 = 0.306$) having the highest weight in this difference.

Regarding the LDA, a forward iterative inclusion of the different parameters has been made to select the color and phenol variables with a greater discrimination, being the lambda of Wilks the criterion for the selection. The LDA analyses have been achieved considering the percentage of pollen of *E. resinifera* if it is <20% or >20%. The variables selected by LDA were gallic acid, vanillic acid, *p*-coumaric acid, quercetin, a^* and L^* (see Table S5). *p*-value < 0.01 has been found in three variables, gallic and *p*-coumaric acids and a^*. The program calculates the canonical variable which best discriminates correlations between the two group of honey samples (according to the percentage of *E. resinifera* pollen <20% or >20%), and their coefficients. In Table S6 we can observe the cumulative proportion of total dispersion as well as the standardized coefficients for the canonical variable. The coordinate a^* and *p*-coumaric acid are the two variables which contribute for the majority of the discrimination between the group of samples. Because that solely two groups have been considered, only one classification

Table 2
Classification matrix of the two group of honey samples according to their percentage of *E. resinifera* pollen.

	% correct	Group a	Group b
a	100	21	0
b	75	2	6
Total	93	23	6

Group a: 21 honey samples with percentage of *E. resinifera* pollen >20%; group b: 8 honey samples with percentage of *E. resinifera* pollen <20%.

function linked to flavonoids, vanillic acid, chrysin (positive sign) and a^*, L^*, *p*-coumaric acid, gallic acid, pinocembrin and quercetin (negative sign), have been carried out, which achieved in a good separation among the honey samples (Fig. 4). In Table 2 it can be see the validity of the Classification functions (data not shown) according to the settlement % of the cases in their corresponding group. We can see that all the samples with percentage of *E. resinifera* pollen > at 20% were accurately assigned into their correct group, while the honey samples with % of *E. resinifera* pollen < at 20% shown lower agreement percentage (<75%).

The use of statistical approach corroborates the effectiveness of the melissopalynological and physicochemical analysis, as phenolic compounds and color parameters, in diagnosing the uniflorality of this honey type. Although the majority of the honey samples were distributed into corresponding group, except for two honey samples, which presented lower concentration of gallic and *p*-coumaric acids. Researches on less studied unifloral honeys should maintain especially those where the pollen % necessary to consider the uniflorality of honey are doubtful.

4. Conclusions

The evaluation of the physicochemical characteristic helps to raise the value of this labeled honey type, from commercial point of view, also, it will assure even more the standing of this unifloral honey in the EU catalog of protected foods (EU Communication 2013/C 232/05, 2013). In the last years, researches on foods that contain phenolic compounds were crucial, and their presence in honey, as markers, allows the authentification of the botanical origin. Thus, in our research the analysis of phenolic acid and flavonoids allows the separation of 17 phenolic compounds, which four of them (syringic acid, ethyl gallate, *m*-coumaric acid and naringenin) could be considered as biomarkers of this honey variety. Also, many correlations between the chromatic attributes and phenolic acids, total phenolic compounds, caffeic acid, *p*- and *m*-coumaric acids and hesperidin have been demonstrated. Lastly, correlation between phenols, color parameters and percentage of pollen of *E. resinifera* has been established.

CRediT authorship contribution statement

Dolores Hernanz: Conceptualization, Supervision, Writing – original draft, &, Funding acquisition. **M. Ángeles Palomar:** Investigation. **Abdelkarim Moujanni:** Investigation, &, Writing – original draft. **Abdelkhalid Essamadi:** Investigation, &, Writing – original draft. **Francisco J. Heredia:** Conceptualization, Supervision, Writing & review. **Anass Terrab:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

Agriculture Ministry of Morocco. (2015). Labelled products in Morocco [on Line] Available in http://www.agriculture.gov.ma/sites/default/files/siam15-produit_terroir-vang.pdf. [Visited at 01.03.2018].

Al-Farsi, M., Al-Amri, A., Al-Hadhrami, A., & Al-Belushi, S. (2018). Color, flavonoids, phenolics and antioxidants of Omani honey. *Heliyon, 4*(10), Article e00874.

Andrade, P., Ferreres, F., & Amaral, M. T. (1997). Analysis of honey phenolic acids by HPLC, its application to honey botanical characterization. *Journal of Liquid Chromatography & Related Technologies, 20*(14), 2281–2288.

Appendino, G., & Szallasi, A. (1997). Euphorbium: Modern research on its active principle, resiniferatoxin, revives an ancient medicine. *Life Sciences, 60*(10), 681–696.

Balcázar-Cruz, L., Valadez-Villarreal, A., López-Naranjo, J. I., Ochoa-Flores, A. A., Rodríguez-Blanco, L., & López-Hernández, E. (2019). Relación del contenido de flavonoides y color en miel de abeja (*Apis mellifera*) originaria del estado de Tabasco, México. *Investigación y Desarrollo en Ciencia y Tecnología de Alimentos, 4*, 818–825.

Bayram, N. E., Canli, D., Gercek, Y. C., Bayram, S., Celik, S., Güzel, F., et al. (2020). Macronutrient and micronutrient levels and phenolic compound characteristics of monofloral honey samples. *Journal of Food & Nutrition Research, 59*(4).

Becerril-Sánchez, A. L., Quintero-Salazar, B., Dublán-García, O., & Escalona-Buendía, H. B. (2021). Phenolic compounds in honey and their relationship with antioxidant activity, botanical origin, and color. *Antioxidants, 10*(11), 1700.

Benlyas, M., Alem, C., & Filali-Zegzouti, Y. (2016). Evaluation of antioxidant, antibacterial and antifungal activities of eleven monofloral honey samples collected from Morocco. *Journal of Chemical and Pharmaceutical Research, 8*(3), 299–306.

Beretta, G., Granata, P., Ferrero, M., Orioli, M., & Facino, R. M. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta, 533*(2), 185–191. https://doi.org/10.1016/j.aca.2004.11.010

Bettar, I., González-Miret, M. L., Hernanz, D., Marconi, A., Heredia, F. J., & Terrab, A. (2019). Characterisation of Moroccan Spurge (Euphorbia) honeys by their physicochemical characteristics, mineral contents and colour. *Arabian Journal of Chemistry, 12*(8), 2052–2060.

Biesaga, M., & Pyrzyńska, K. (2013). Stability of bioactive polyphenols from honey during different extraction methods. *Food Chemistry, 136*(1), 46–54. https://doi.org/10.1016/j.foodchem.2012.07.095

Boutoub, O., El-Guendouz, S., Manhita, A., Dias, C. B., Estevinho, L. M., Paula, V. B., et al. (2021). Comparative study of the antioxidant and enzyme inhibitory activities of two types of Moroccan *Euphorbia* entire honey and their phenolic extracts. *Foods, 10*(8), 1909.

Cabrera, M., Perez, M., Gallez, L., Andrada, A., & Balbarrey, G. (2017). Colour, antioxidant capacity, phenolic and flavonoid content of honey from the Humid Chaco Region, Argentina. *Phyton, 86*, 124.

Campillo, N., Viñas, P., Férez-Melgarejo, G., & Hernández-Córdoba, M. (2015). Dispersive liquid–liquid microextraction for the determination of flavonoid aglycone compounds in honey using liquid chromatography with diode array detection and time-of-flight mass spectrometry. *Talanta, 131*, 185–191.

Campone, L., Piccinelli, A. L., Pagano, I., Carabetta, S., Di Sanzo, R., Russo, M., et al. (2014). Determination of phenolic compounds in honey using dispersive liquid-liquid microextraction. *Journal of Chromatography A, 1334*, 9–15. https://doi.org/10.1016/j.chroma.2014.01.081

Ciulu, M., Spano, N., Pilo, M. I., & Sanna, G. (2016). Recent advances in the analysis of phenolic compounds in unifloral honeys. *Molecules, 21*(4), 451.

Daci-Ajvazi, M., Mehmeti, A., Zeneli, L., & Daci, N. (2017). Evaluation of antioxidant activity, heavy metals and colour intensity of honeys from different parts of Kosovo. *Journal of Environmental Protection and Ecology, 18*, 737–748.

Dimitrova, B., Gevrenova, R., & Anklam, E. (2007). Analysis of phenolic acids in honeys of different floral origin by solid-phase extraction and high-performance liquid chromatography. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques, 18*(1), 24–32. https://doi.org/10.1002/pca.948

Escuredo, O., Rodríguez-Flores, M. S., Rojo-Martínez, S., & Seijo, M. C. (2019). Contribution to the chromatic characterization of unifloral honeys from Galicia (NW Spain). *Foods, 8*(7), 233. https://doi.org/10.3390/foods8070233

EU Communication 2013/C 232/05. (2013). Indications géographiques du Royaume du Maroc. *Journal officiel de l'Union Européenne, 2013*, 13–16. *C 232* – 10.08.

Fattorusso, E., Lanzotti, V., Taglialatela-Scafati, O., Tron, G. C., & Appendino, G. (2002). Bisnorsesquiterpenoids from *Euphorbia resinifera* Berg. and an expeditious procedure to obtain resiniferatoxin from its fresh latex. *European Journal of Organic Chemistry, 2002*(1), 71–78.

Ferreira, I. C., Aires, E., Barreira, J. C., & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry, 114*, 1438–1443. https://doi.org/10.1016/j.foodchem.2008.11.028

Gabe, V., Kacergius, T., Abu-Lafi, S., Kalesinskas, P., Masalha, M., Falah, M., et al. (2019). Inhibitory effects of ethyl gallate on *Streptococcus mutans* biofilm formation by optical profilometry and gene expression analysis. *Molecules, 24*(3), 529. https://doi.org/10.3390/molecules24030529

Gašić, U. M., Natić, M. M., Mišić, D. M., Lušić, D. V., Milojković-Opsenica, D. M., Tešić, Ž. L., et al. (2015). Chemical markers for the authentication of unifloral *Salvia officinalis* L. honey. *Journal of Food Composition and Analysis, 44*, 128–138.

Gheldof, N., Wang, X. H., & Engeseth, N. J. (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry, 50*(21), 5870–5877.

Gómez-Caravaca, A. M., Gómez-Romero, M., Arráez-Román, D., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2006). Advances in the analysis of phenolic compounds in products derived from bees. *Journal of Pharmaceutical and Biomedical Analysis, 41*(4), 1220–1234.

Gonzales, A. P., Burin, L., & del Pilar Buera, M. (1999). Color changes during storage of honeys in relation to their composition and initial color. *Food Research International, 32*(3), 185–191.

González-Miret, M. L., Terrab, A., Hernanz, D., Fernández-Recamales, M.Á., & Heredia, F. J. (2005). Multivariate correlation between color and mineral composition of honeys and by their botanical origin. *Journal of Agricultural and Food Chemistry, 53*(7), 2574–2580.

Heredia, F. J., Álvarez, C., González-Miret, M. L., & Ramírez, A. (2004). *CromaLab®, Análisis de color. Registro General de la Propiedad Intelectual SE-1052-04* (Sevilla, Spain).

Karabagias, I. K., Halatsi, E. Z., Karabournioti, S., Kontakos, S., & Kontominas, M. G. (2017). Impact of physicochemical parameters, pollen grains, and phenolic compounds on the correct geographical differentiation of fir honeys produced in Greece as assessed by multivariate analyses. *International Journal of Food Properties, 20*, S520–S533. https://doi.org/10.1080/10942912.2017.1300811

Karabagias, I. K., Vavoura, M. V., Nikolaou, C., Badeka, A. V., Kontakos, S., & Kontominas, M. G. (2014). Floral authentication of Greek unifloral honeys based on the combination of phenolic compounds, physicochemical parameters and chemometrics. *Food Research International, 62*, 753–760.

Kečkeš, S., Gašic, U., Velickovic, T. C., Milojkovic-Opsenica, D., Natic, M., & Tešic, Z. (2013). The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry. *Food Chemistry, 138*(1), 32–40. https://doi.org/10.1016/j.foodchem.2012.10.025

Khiati, B., Bacha, S., Ahmed, M., Aissat, S., Meslem, A., & Djebli, N. (2013). Wound care with euphorbia honey after nucleation: A case report. *Clinical Microbiology: Open Access*. https://doi.org/10.4172/2327-5073.1000129

Kıvrak, Ş., & Kıvrak, İ. (2017). Assessment of phenolic profile of Turkish honeys. *International Journal of Food Properties, 20*(4), 864–876.

Leong, M. S., & Copenhaver, D. (2018). *Potent neurotoxins for cancer pain treatment: Resiniferatoxin and Saporin* (2nd ed.). Academic Press Edition. Neuromodulation: Comprehensive Textbook of Principles, Technologies, and Therapies.

Michalkiewicz, A., Biesaga, M., & Pyrzynska, K. (2008). Solid-phase extraction procedure for determination of phenolic acids and some flavonols in honey. *Journal of Chromatography A, 1187*(1–2), 18–24.

Moniruzzaman, M., Sulaiman, S. A., Khalil, M. I., & Gan, S. H. (2013). Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: A comparison with manuka honey. *Chemistry Central Journal, 7*(1), 1–12. https://doi.org/10.1186/1752-153X-7-138

Pascual-Maté, A., Osés, S. M., Fernández-Muiño, M. A., & Sancho, M. T. (2018). Analysis of polyphenols in honey: Extraction, separation and quantification procedures. *Separation and Purification Reviews, 47*(2), 142–158.

Pasupuleti, V. R., Sammugam, L., Ramesh, N., & Gan, S. H. (2017). Honey, propolis, and royal jelly: A comprehensive review of their biological actions and health benefits. *Oxidative Medicine and Cellular Longevity, 2017*, 1259510. https://doi.org/10.1155/2017/1259510

Perna, A., Intaglietta, I., Simonetti, A., & Gambacorta, E. (2013). A comparative study on phenolic profile, vitamin C content and antioxidant activity of Italian honeys of different botanical origin. *International Journal of Food Science and Technology, 48*(9), 1899–1908.

Pontis, J. A., Costa, L. A. M. A. D., Silva, S. J. R. D., & Flach, A. (2014). Color, phenolic and flavonoid content, and antioxidant activity of honey from Roraima, Brazil. *Food Science and Technology, 34*, 69–73.

Puścion-Jakubik, A., Karpińska, E., Moskwa, J., & Socha, K. (2022). Content of phenolic acids as a marker of polish honey varieties and relationship with selected honey-quality-influencing variables. *Antioxidants, 11*(7), 1312.

Rebiai, A., & Lanez, T. (2014). Comparative study of honey collected from different flora of Algeria. *Journal of Fundamental and Applied Sciences, 6*(1), 48–55.

Ricciardelli D'Albore, G. (1998). *Mediterranean Melissopalynology*. Perugia: Instituto di Entomologia agraria, Universitá degli studi di Perugia.

Rosa, A., Tuberoso, C. I. G., Atzeri, A., Melis, M. P., Bifulco, E., & Dessì, M. A. (2011). Antioxidant profile of strawberry tree honey and its marker homogentisic acid in several models of oxidative stress. *Food Chemistry, 129*(3), 1045–1053.

Scripcă, L. A., Norocel, L., & Amariei, S. (2019). Comparison of physicochemical, microbiological properties and bioactive compounds content of grassland honey and other floral origin honeys. *Molecules, 24*(16), 2932. https://doi.org/10.3390/molecules24162932

Shafiee, S., Minaei, S., Moghaddam-Charkari, N., Ghasemi-Varnamkhasti, M., & Barzegar, M. (2013). Potential application of machine vision to honey characterization. *Trends in Food Science & Technology, 30*(2), 174–177.

Smetanska, I., Alharthi, S. S., & Selim, K. A. (2021). Physicochemical, antioxidant capacity and color analysis of six honeys from different origin. *Journal of King Saud University Science, 33*(5), Article 101447.

Stephens, J. M., Schlothauer, R. C., Morris, B. D., Yang, D., Fearnley, L., Greenwood, D. R., et al. (2010). Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. *Food Chemistry, 120*(1), 78–86. https://doi.org/10.1016/j.foodchem.2009.09.074

Stinco, C. M., Benítez-González, A. M., Hernanz, D., Vicario, I. M., & Meléndez-Martínez, A. J. (2014). Development and validation of a rapid resolution liquid chromatography method for the screening of dietary plant isoprenoids: Carotenoids, tocopherols and chlorophylls. *Journal of Chromatography A, 1370*, 162–170.

Stinco, C. M., Benítez-González, A. M., Meléndez-Martínez, A. J., Hernanz, D., & Vicario, I. M. (2019). Simultaneous determination of dietary isoprenoids (carotenoids, chlorophylls and tocopherols) in human faeces by Rapid Resolution Liquid Chromatography. *Journal of Chromatography A, 1583*, 63–72.

Terrab, A., Díez, M. J., & Heredia, F. J. (2003). Palynological, physico-chemical and colour characterization of Moroccan honeys: I. River red gum (*Eucalyptus camaldulensis* dehnh) honey. *International Journal of Food Science and Technology, 38*(4), 379–386.

Terrab, A., Escudero, M. L., González-Miret, M. L., & Heredia, F. J. (2004). Colour characteristics of honeys as influenced by pollen grain content: A multivariate study. *Journal of the Science of Food and Agriculture, 84*(4), 380–386.

Terrab, A., González-Miret, L., & Heredia, F. J. (2004). Colour characterisation of thyme and avocado honeys by diffuse reflectance spectrophotometry and spectroradiometry. *European Food Research and Technology, 218*(5), 488–492.

Terrab, A., Moujanni, A., Essamadi, A. K., Hernanz, D., Díez, M. J., & Berjano, R. (2022). A palynological and geographical characterization of labeled resin spurge honey: *Euphorbia resinifera*. *Palynology, 46*(1), 1–10.

The National Honey Board. (2003). *Honey-health and therapeutic qualities. 390 Lashley street longmont*. www.nhb.org.

Tomás-Barberán, F. A., Ferreres, F., Blázquez, M. A., Garcia-Viguera, C., & Tomás-Lorente, F. (1993). High-performance liquid chromatography of honey flavonoids. *Journal of Chromatography A, 634*(1), 41–46. https://doi.org/10.1016/0021-9673

Tomás-Barberán, F. A., Martos, I., Ferreres, F., Radovic, B. S., & Anklam, E. (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture, 81*, 485–496.

Trautvetter, S., Koelling-Speer, I., & Speer, K. (2009). Confirmation of phenolic acids and flavonoids in honeys by UPLC-MS. *Apidologie, 40*(2), 140–150.

Tuberoso, C. I., Bifulco, E., Jerkovic, I., Caboni, P., Cabras, P., & Floris, I. (2009). Methyl syringate: A chemical marker of asphodel (*Asphodelus microcarpus* Salzm. Et Viv.) monofloral honey. *Journal of Agricultural and Food Chemistry, 57*(9), 3895–3900.

Wabaidur, S. M., Ahmed, Y. B. H., Alothman, Z. A., Obbed, M. S., Al-Harbi, N. M., & Al-Turki, T. M. (2015). Ultra high performance liquid chromatography with mass spectrometry method for the simultaneous determination of phenolic constituents in honey from various floral sources using multiwalled carbon nanotubes as extraction sorbents. *Journal of Separation Science, 38*(15), 2597–2606.

Wang, S., Liang, H., Zhao, Y., Wang, G., Yao, H., Kasimu, R., et al. (2016). New triterpenoids from the latex of *Euphorbia resinifera* Berg. *Fitoterapia, 108*, 33–40. https://doi.org/10.1016/j.fitote.2015.11.009

Wilczyńska, A. (2014). Effect of filtration on colour, antioxidant activity and total phenolics of honey. *LWT–Food Science and Technology, 57*(2), 767–774.

Zhao, J., Du, X., Cheng, N., Chen, L., Xue, X., Wu, L., et al. (2016). Identification of monofloral honeys using HPLC–ECD and chemometrics. *Food Chemistry, 194*, 167–174.