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Quality assessment of Portuguese monofloral honeys. Physicochemical parameters as tools in botanical source differentiation

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ABSTRACT

The quality evaluation and physicochemical parameters assessment of Portuguese monofloral honeys were performed. Fifty-one monofloral honeys were collected from several regions from mainland Portugal, and from the Azores islands, producer labelled as carob tree (n = 5), chestnut (n = 2), eucalyptus (n = 5), bell heather (n = 6), incense (n = 4), lavender (n = 8), orange (n = 9), rape (n = 2), raspberry (n = 2), rosemary (n = 1), sunflower (n = 3), and strawberry tree (n = 4). Pollen analysis and microbiological safety were evaluated, and the parameters such as colour index, moisture content, electrical conductivity, hydroxymethylfurfural, pH, free and total acidity, diastase activity, proline, and sugar profile were assessed for physicochemical characterization, in all 51 monofloral honeys. After melissopalynological examination, the honeys were either confirmed as monofloral, or classified as multifloral with predominance of a specific pollen type or multifloral. Microbiological analysis showed that honeys were safe for human consumption. Pairwise comparisons of physicochemical parameters, using only honey types with $n \geq 3$, revealed significant differences between honey types. Despite some homogeneity in sugar profile among honeys, eucalyptus honey was significantly different in glucose, maltose and maltulose content compared to incense, orange and sunflower honeys, and also exhibited a higher isomaltose amount compared to all analyzed honeys. Electrical conductivity, colour index, free and total acidity, and diastase activity showed significant differences between the analyzed honeys, indicating that these parameters may provide an additional tool in monofloral honey identification.

1. Introduction

Honey has been consumed since ancient times due to its nutritional and health benefits, being nowadays widely used in many areas of food and pharmaceutical industries (Soares et al., 2017; Karapetsas et al., 2020).

This product contains several carbohydrates, mainly fructose and glucose, and other minor constituents like proteins, enzymes, minerals, vitamins, volatiles, phenolic compounds such as flavonoids, and organic acids (da Silva et al., 2016; Hossain et al., 2021). Honey composition depends remarkably on the botanical source and/or geographic origin (Escuredo et al., 2014), which involves climatic conditions, soil composition, flora variety and the intensity of nectar flow. Other factors that might affect honey's composition include beekeepers' handling, packing procedure, time of and storage's conditions (Thrasyvoulou et al., 2018). The quality of honey is mainly determined by sensorial, microbiological analysis and physicochemical parameters comprising moisture content, sugars content, electrical conductivity, diastase

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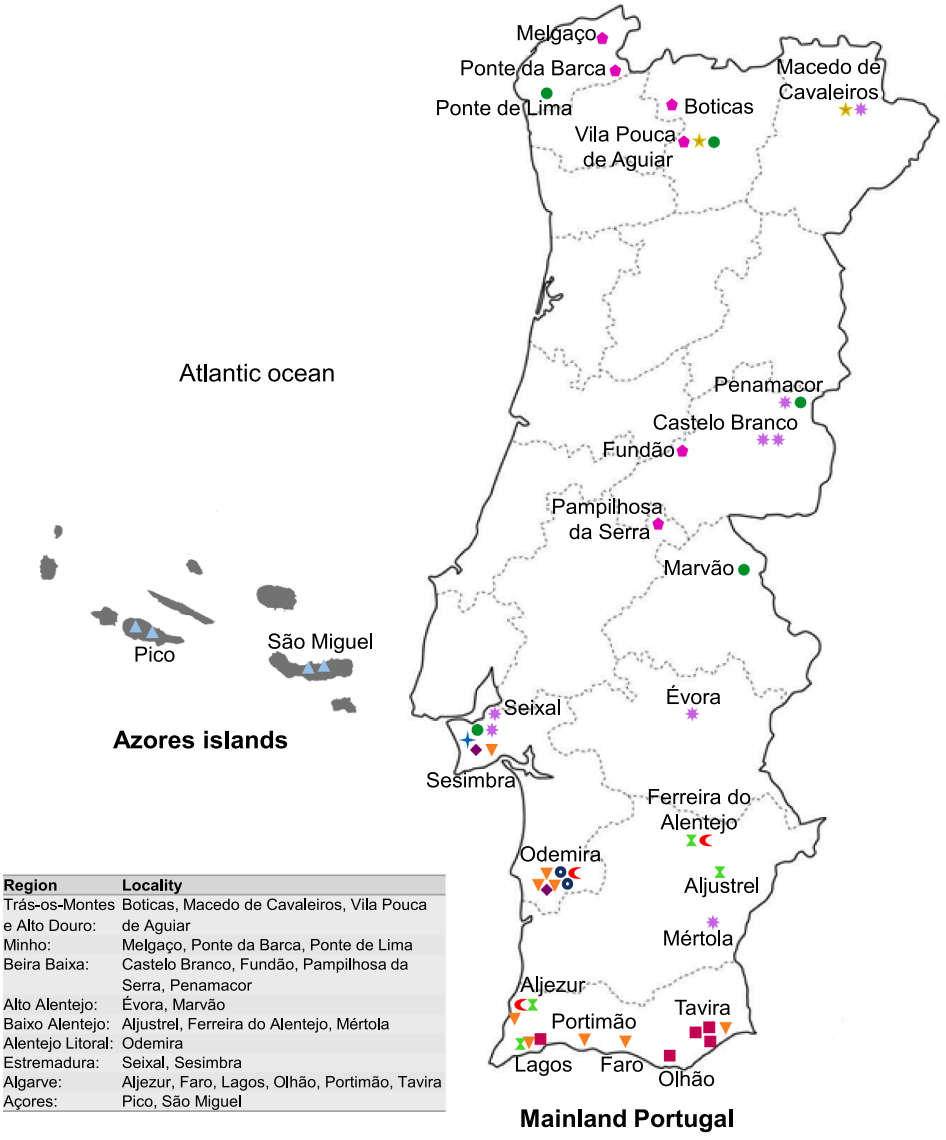
activity (DA) or hydroxymethylfurfural (HMF) determination (Bogdanov et al., 2002). Honey's natural acidity, low protein content and high viscosity, contribute to decrease the amount of atmospheric oxygen penetration, causing a low probability of pathogen presence (Sinacori et al., 2014). However, honey is not a sterile product due to two main sources of contamination, with the first including pollen, nectar, digestive tracts of honeybees, dust, air, and soil, which are very difficult to control. Secondary sources comprising people's honey manipulation, food handlers, cross-contamination, equipment and buildings, can be controlled applying good manufacturing practices (Finola et al., 2007).

According to the European Union (EU) quality policy, and EU Database of Origin & Registration (DOOR, 2021), honeys from a specific region and following a traditional production process may be identified with the labels Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI). Portugal has a great richness and diversity of melliferous flora all over the country, including the Azores islands, which enables the production of several monofloral honeys, namely bell heather (*Erica* spp.), eucalyptus (*Eucalyptus* spp.), incense (*Pittosporum undulatum*), orange (*Citrus* spp.), lavender (*Lavandula stoechas*, *L. pedunculata*, *L. luisieri*) or viper's bugloss (*Echium* spp.) honeys. Currently, Portugal is the country with the highest number of honeys registered in the EU (nine PDO honeys), followed by Spain (five PDO and one PGI honeys) and France (two PDO and three PGI honeys) (DOOR, 2021). Monofloral honey, mainly produced from a single botanical source (generally at least 45% of the total pollen grains), in opposition to multifloral, is characterised by having highly distinguishing aromas, which could indicate the presence of specific volatile compounds (Soares et al., 2017). Traditionally, pollen analysis or mellisopalynology is the method used to achieve botanical source and geographic origin (Von der Ohe et al., 2004; Rodopoulou et al., 2021), remaining nowadays as the reference method despite demanding a very experienced analyst. To overcome this requirement other approaches are applied to complement pollen examination, such as physicochemical analysis, chromatographic, spectroscopic or molecular methods (Soares et al., 2017; Anjos et al., 2018).

Considering the reduced number of studies on Portuguese monofloral honeys (Mendes et al., 1998; Estevinho et al., 2012; Alves et al., 2013; Tomás et al., 2017; Karabagias et al., 2018), the consumers growing interest in these honey types, the necessity of ensuring their quality and authenticity, and the social and economic relevance of this country product, it is highly important to gain further knowledge on them, to establish the value of Portuguese honeys in competing and



Region	Locality
Trás-os-Montes e Alto Douro:	Boticas, Macedo de Cavaleiros, Vila Pouca de Aguiar
Minho:	Melgaço, Ponte da Barca, Ponte de Lima
Beira Baixa:	Castelo Branco, Fundão, Pampilhosa da Serra, Penamacor
Alto Alentejo:	Évora, Marvão
Baixo Alentejo:	Aljustrel, Ferreira do Alentejo, Mértola
Alentejo Litoral:	Odemira
Estremadura:	Seixal, Sesimbra
Algarve:	Aljezur, Faro, Lagos, Olhão, Portimão, Tavira
Açores:	Pico, São Miguel

Fig. 1. Identification of the mainland Portugal and Azores archipelago geographical origin, and number, of the studied honey samples (n = 51), identified according to monofloral honey type. Carob tree (■, n = 5), chestnut (★, n = 2), eucalyptus (●, n = 5), bell heather (⬟, n = 6), incense (▲, n = 4), Lavender (✱, n = 8), orange (▼, n = 9), rape (◘, n = 2), raspberry (◆, n = 2), rosemary (✦, n = 1), sunflower (◖, n = 3), strawberry tree (⧗, n = 4). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

demanding markets. In view of this, and using fifty-one honeys of labelled monofloral honeys of carob tree (*Ceratonia siliqua*), chestnut (*Castanea sativa*), eucalyptus (*Eucalyptus* spp.), bell heather (*Erica* spp.), incense (*Pittosporum undulatum*), lavender (*Lavender* spp.), orange (*Citrus* spp.), rape (*Brassica* spp.), raspberry (*Rubus* spp.), rosemary (*Rosmarinus officinalis*), sunflower (*Helianthus annuus*), and strawberry tree (*Arbutus unedo*), the main purposes of the present study were: a) to contribute to the knowledge on monofloral honeys, regarding pollen analysis, microbiological safety, and physicochemical parameters (colour index, moisture content, electrical conductivity, hydroxymethylfurfural, pH, free and total acidity, diastase activity, proline, and sugar profile), and b) to assess which parameters contribute the most to the honey's differentiation according to the botanical source.

2. Material and methods

2.1. Honey sampling

Fifty-one honey glass containers representative of Portuguese monofloral honey types were collected in several regions of the mainland Portugal (Minho, Trás-os-Montes e Alto Douro, Beira Baixa, Estremadura, Alto Alentejo, Baixo Alentejo, Alentejo Litoral and Algarve), and of the Azores islands (São Miguel and Pico) (Fig. 1). Most honeys were obtained from local producers, and the remaining from specialized shops, between 2015 and 2018. Monofloral honeys were codified based on their label botanical source: five of carob tree (Ct1-Ct5), two of chestnut (C1-C2), five of eucalyptus (E1-E5), six of bell heather (H1-H6), four of incense (I1-I4), eight of lavender (L1-L8), nine of orange (O1-O9), two of rape (R1-R2), two of raspberry (Rb1-Rb2), one of rosemary (Ros1), three of sunflower (Sf1-Sf3) and four of strawberry tree (St1-St4) (Fig. 1).

From each honey glass container three independent samples were collected, and analyzed separately, in a total of 153 samples (Table 1). The honeys were stored in a cool, dry place until further assessment. All the honeys were subjected to pollen analysis to confirm their botanical source.

2.2. Melissopalynological analysis

Qualitative pollen analysis was performed according to the acetolytic method as described by Louveaux et al. (1978). The pollen was examined using an optic microscope (Nikon Microphot - FXA) at 40x and 100x. Pollen grains were grouped into pollen types, with similar morphology, normally being produced from plants of the same genus. For an estimation of the relative frequencies of pollen types, a minimum of 600 pollen grains were counted per sample.

The relative frequency, expressed as a percentage of the dominant identified pollen type was considered for classification of the honeys in monofloral or multifloral, according to their botanical source. Honey's obtained from eucalyptus, bell heather, lavender, orange, rape, rosemary, sunflower and strawberry tree were classified according to Von der Ohe et al. (2004), of chestnut as mentioned in Perez-de-Zabalza (1992), carob tree and raspberry honey's botanical source were established according to dominant pollen type (≥45%). Azores' incense honey was classified according to the Commission Delegated Regulation (EU) PDO-PT-0268-AM01 (2019).

2.3. Microbiological quality

The microbiological quality assessment of the fifty-one honeys followed national and international standards, namely NP 4405:2002 for aerobic mesophilic bacteria, ISO 21527–2:2008 for yeasts and moulds, ISO 21528–2:2017 for Enterobacteriaceae and ISO 15213:2003 for sulphite-reducing *Clostridium* spp. Detection of *Escherichia coli* strain was also included in the tests, following the Portuguese Pharmacopoeia (Farmacopeia Portuguesa 9, 2008). Briefly, ten grams of each sample were transferred to 90 mL of peptone water and homogenized. Decimal dilutions were prepared using the same diluent. Microbial counts were expressed in Log10 colony-forming units (CFU)/g. The microbiological determinations were done in duplicate.

2.4. Physicochemical determinations

The colour index, moisture, electrical conductivity, HMF content, pH, free and total acidity, DA, and proline content were analyzed using the procedures recommended by the International Honey Commission (Bogdanov et al., 2002). All the determinations were performed in triplicate for each sample. Colour index was determined in a C221 colorimeter (Hanna Instruments, Woonsocket, RI, USA), moisture content was determined using a hand refractometer (Digit-5890, Ref: 8100.5890), electrical conductivity was measured with a calibrated Consort C868 conductivity meter (Hanna Instruments, Woonsocket, RI, USA), pH, free and total acidity measurements were performed using a HI902 potentiometer titrator (Hanna instruments, pH 211 microprocessor pH meters), and absorbance measures of DA and HMF and proline content were performed using a UV/Vis spectrophotometer (Specord 200 spectrophotometer, Analytikjena, Jena, Germany).

Sugar composition was analyzed by high performance liquid chromatography coupled with a refractive index detector (HPLC-RI) according to Tomás et al. (2017). HPLC-RI was performed on an integrated

Table 1
Number of glass containers per honey type, number of samples analyzed per honey type for physicochemical parameters evaluation and number of glass containers per honey type used in statistical analysis.

Analyzed honey types	Number of glass containers from different producers per honey type	Number of samples per honey type for PP evaluation	Number of glass containers per honey type for statistical analysis
Carob tree	5	15	3
Chestnut	2	6	NA
Eucalyptus	5	15	3
Bell heather	6	18	4
Incense	4	12	4
Lavender	8	24	5
Orange	9	27	6
Rape	2	6	NA
Raspberry	2	6	NA
Rosemary	1	3	NA
Sunflower	3	9	3
Strawberry tree	4	12	3
Total	51	153	31

PP: Physicochemical parameters. NA: Not applicable.

Knauer system with pump (Smartline 1000), with a degasser (Smartline 5000), a UV detector (Knauer Smartline 2300) and an autosampler (Jasco, AS-2057). Data acquisition and remote control of the HPLC system was done by Clarity Chrom software (Knauer, Berlin, Germany). The sugar profile was obtained by comparing the retention time of samples and the standard individual sugar solutions. Using standard samples of known composition and concentration, calibration curves were determined, as follows: fructose (3.1–60.0 mg/mL, $y = 1.6767x - 0.0552$, $R^2 = 0.999$), glucose (2.3–45.0 mg/mL, $y = 1.5283x - 0.2337$, $R^2 = 0.999$), isomaltose (0.4–6.9 mg/mL, $y = 0.6013x + 0.0993$, $R^2 = 0.999$), maltose (0.2–4.5 mg/mL, $y = 1.1554x + 0.0555$, $R^2 = 0.993$), maltulose (0.2–4.5 mg/mL, $y = 1.3132x + 0.012$, $R^2 = 0.998$), melibiose (0.5–4.5 mg/mL, $y = 0.8321x - 0.1062$, $R^2 = 0.998$), sucrose (0.8–15 mg/mL, $y = 1.678x - 0.0489$, $R^2 = 0.999$), trehalose (0.2–4.5 mg/mL, $y = 1.6215x - 0.0141$, $R^2 = 0.998$), turanose (0.2–2.8 mg/mL; $y = 1.4418x - 0.1113$, $R^2 = 0.999$), erlose (0.1–1.7 mg/mL, $y = 0.6721x + 0.094$, $R^2 = 0.996$), melezitose (0.5–4.5 mg/mL, $y = 1.42x - 0.013$, $R^2 = 0.999$) and raffinose (0.5–4.5 mg/mL, $y = 1.3844x - 0.1626$, $R^2 = 0.999$). The assay was performed in triplicate for each honey glass container and the results were expressed as g / 100 g of honey, dry mass.

2.5. Statistical analysis

To deal with the presence of repeated measures in the evaluation of the effects of botanical source on the physicochemical parameters, linear regression models were estimated using generalized estimation equations (GEE), assuming Gaussian distribution of the parameters under evaluation. Statistical analyses were performed in R (Version 4.0.2) and RStudio (Version 1.3.1093) environments. Package gee (Generalized Estimation Equation Solver, 2012) was used to estimate the models. *Post hoc* analysis following model's estimation consisted in pairwise comparisons to identify significant differences between honeys from different botanical source. In these procedures family wise type I error was controlled using Bonferroni's correction. The statistical comparison between honey types has been conducted with monofloral honeys and with predominance of a specific pollen type honeys, after pollen analysis, with three or more glass containers from different producers per honey type, according to botanical source. Differences with $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Pollen analysis

Before assessing the physicochemical parameters, pollen analysis (PA) was performed on all fifty-one honeys to ascertain the correspondence between the monofloral honey label and the frequency of the most abundant pollen type(s), represented in Table 2 and Fig. 2.

Overall, and depending on the honey type, 53% of the total analyzed honeys confirmed the monofloral honey label, after melissopalynological analysis (Fig. 2). Melissopalynological analysis enabled the identification of three main groups of honeys, one of which with subgroups: a) honeys whose pollen analysis confirmed the monofloral label type, b) honeys whose percentage of the dominant pollen type was not enough to classify them as monofloral, therefore being considered multifloral, b1) honeys whose percentage of the dominant pollen was close to the minimum limit to be monofloral (7–30%, being this last value linked to eucalyptus honey), were considered multifloral with predominance of a specific pollen type and c) honey whose botanical source was reassigned, as pollen analysis showed a predominance of a pollen type different to that indicated in the label. The honeys that matched the monofloral honey label, were marked in bold in Table 2. Monofloral honeys whose pollen analysis showed values below the expected standard were further treated as multifloral, or multifloral with predominance of a specific pollen type (Table 2).

Ceratonia siliqua pollen (Fig. 2) frequency in labelled carob tree honeys varied between 28 and 75%. The minimum percentage of *Ceratonia siliqua* pollen required to classify a honey as carob tree monofloral honey is not yet officially established, so this study considered 45% as the minimum limit, as described by Terrab et al. (2003) for Moroccan honeys. Nevertheless, Ferrauto & Pavone (2013), considered 15% enough to classify similar type Sicilian honeys as monofloral.

The pollen content in chestnut honeys (Fig. 2) showed a frequency of *Castanea sativa* pollen type of 31% and 80% for C1 and C2 honeys respectively. Nevertheless, only sample C2 may be considered as chestnut monofloral honey, based on chestnut pollen frequency reported by Perez-de-Zabalza (1992) 77%, and Escuredo et al. (2014) 78%, for this type of honey.

The five analyzed eucalyptus honeys showed a wide variation in *Eucalyptus* spp. pollen frequency (8–88%). Using the criteria of Von der Ohe et al. (2004), of > 83% of *Eucalyptus* ssp. pollen to classify as monofloral, only sample E3 with 88% of this pollen type, matched the criteria (Fig. 2). However, other authors classify as monofloral if the dominant pollen reaches 70% (Feás et al., 2010). *Castanea* spp. and *Eucalyptus* spp. pollen grains are strongly over-represented in the pollen spectra in chestnut and eucalyptus honeys (Oddo and Piro, 2004; Von der Ohe et al., 2004).

The frequency of *Erica* spp. pollen in bell heather honeys, ranged between 8 and 52% and only H2 and H4 could be considered as monofloral honeys, again using the criteria of pollen frequency (>45%) defined by Von der Ohe et al. (2004). These results are in accordance with the common representativeness of *Erica* spp. pollen in bell heather honeys, though some authors refer 45% as too high, being hardly reached due to the morphology of *Erica*'s pollen grains as well to its dispersion in tetrads (Escuredo et al., 2014).

Incense honey classified as PDO is only produced in the Azores islands (Fig. 1), thus studies related with this honey type remain scarce. According to the Commission Delegated Regulation (EU) PDO-PT-0268-AM01 (2019) *Pittosporum undulatum* pollen grains (Fig. 2) should reach at least 30% in incense honey. In this study, all four honeys fitted in these criteria showing a *Pittosporum undulatum* pollen frequency of 34–51%.

Lavender honey, like strawberry tree or citrus honeys, contains under-represented pollen grains and the botanical classification must be achieved with a lower pollen frequency (15%). In this study the frequency of *Lavandula* spp. pollen ranged between 5 and 43% (Fig. 2).

Citrus spp. pollen type in orange honeys ranged from 1 to 81% (Fig. 2). Similar high pollen frequency values, 1–88%, were reported in Spanish orange honeys (Juan-Borrás et al., 2015). *Citrus* spp. pollen is another under-represented pollen in honeys, and in this study 10% was the minimum limit to ascertain the botanical source of orange honey. The range of pollen values sometimes found is dependent on the different *Citrus* species and cultivars (Oddo and Piro, 2004).

The two honeys labelled as rape honey showed a *Brassica* spp. pollen range from 28 to 45%. According to Von der Ohe et al. (2004), *Brassica* spp. pollen should be ≥ 60% to label rape honey as monofloral, thus the analyzed rape honeys were classified as multifloral, despite other authors considering a lower limit value of 45% to classify this honey as monofloral (Pauliuc et al., 2020).

Only raspberry honey Rb2 was considered monofloral using Von der Ohe et al. (2004) criteria of presence of ≥ 53% of *Rubus* spp. pollen. This pollen type is also characteristic of blackberry honey, also produced in Portugal.

Rosemary honey sample Ros1 was not considered monofloral since it only contained 1% of *Rosmarinus officinalis* pollen type, unfulfilling the minimum of 10% required according to Louveaux et al. (1978).

The three sunflower honeys analyzed, showed *Helianthus annuus* pollen ranging 13–58%, reaching the minimum of 12% to be considered as monofloral according to Von der Ohe et al. (2004) (Fig. 2).

Arbutus unedo pollen type (Fig. 2) is usually under-represented in strawberry tree honey with a frequency range of 8–20% (Von der Ohe

Table 2
Physicochemical parameters (colour, moisture, electrical conductivity, HMF content, pH, free acidity, total acidity, DA, and proline) of monofloral and multifloral honeys or with predominance of a specific pollen type according to botanical source after melissopalynological analysis.

AH	PA (%)	Botanical Source	Electrical conductivity (mS/cm)	Colour (mm Pfund)	Moisture (%)	HMF (mg/kg)	pH	Free acidity (meq/kg)	Total acidity (meq/kg)	DA (schade number)	Proline (mg/g)
Carob tree											
Ct1	75	*C. siliqua*	0.6 ± 0.0	106 ± 1	14.8 ± 0.0	27.0 ± 3.0	4.1 ± 0.1	25.1 ± 0.1	38.3 ± 0.2	23.2 ± 0.7	1.8 ± 0.6
Ct2	28	Multifloral	0.4 ± 0.0	150 ± 0	15.5 ± 0.0	31.1 ± 6.3	3.7 ± 0.0	19.8 ± 0.2	36.0 ± 0.4	14.0 ± 0.5	1.4 ± 0.2
Ct3	49	*C. siliqua*	0.7 ± 0.0	107 ± 1	15.1 ± 0.1	34.5 ± 1.8	4.4 ± 0.0	20.8 ± 0.2	31.9 ± 0.2	17.9 ± 1.3	1.8 ± 0.5
Ct4	63	*C. siliqua*	0.6 ± 0.0	118 ± 2	16.1 ± 0.1	33.8 ± 1.2	4.1 ± 0.0	24.6 ± 0.4	38.1 ± 0.2	16.7 ± 0.4	1.5 ± 0.1
Ct5	30	Multifloral	1.1 ± 0.0	147 ± 3	16.8 ± 0.0	6.7 ± 1.3	4.6 ± 0.0	29.8 ± 0.7	43.4 ± 0.8	18.1 ± 0.2	1.2 ± 0.1
Chestnut											
C1	31	Multifloral	0.4 ± 0.0	83 ± 2	15.2 ± 0.0	11.9 ± 0.4	4.2 ± 0.2	19.8 ± 0.2	36.5 ± 0.4	27.5 ± 1.2	2.2 ± 0.2
C2	80	*Castanea sativa*	0.8 ± 0.0	147 ± 1	16.2 ± 0.0	5.8 ± 0.4	4.4 ± 0.0	11.7 ± 0.2	24.1 ± 0.5	11.6 ± 0.4	0.9 ± 0.1
Eucalyptus											
E1	68	Multifloral*[1]	0.4 ± 0.0	104 ± 0	16.2 ± 0.1	18.1 ± 1.0	4.0 ± 0.0	14.7 ± 0.2	28.5 ± 0.3	5.4 ± 0.4	0.5 ± 0.1
E2	56	Multifloral*[1]	0.5 ± 0.0	113 ± 1	15.3 ± 0.1	27.7 ± 5.7	4.1 ± 0.1	16.9 ± 0.4	34.1 ± 0.3	9.1 ± 0.1	1.1 ± 0.1
E3	88	*Eucalyptus* spp.	0.4 ± 0.0	75 ± 1	16.3 ± 0.0	35.3 ± 3.5	4.0 ± 0.0	17.2 ± 0.3	30.3 ± 0.2	14.1 ± 0.4	1.2 ± 0.1
E4	45	Multifloral	0.3 ± 0.0	85 ± 1	16.0 ± 0.0	15.1 ± 0.6	3.8 ± 0.1	16.1 ± 0.3	31.5 ± 0.3	5.7 ± 0.4	0.9 ± 0.1
E5	8	Multifloral	0.4 ± 0.0	85 ± 2	16.0 ± 0.0	59.1 ± 5.1	3.7 ± 0.0	22.2 ± 0.0	41.3 ± 0.2	11.0 ± 0.4	1.3 ± 0.1
Bell heather											
H1	36	Multifloral*[2]	0.5 ± 0.0	134 ± 1	16.5 ± 0.1	32.0 ± 5.6	4.1 ± 0.0	23.4 ± 0.5	38.0 ± 0.7	9.2 ± 0.4	0.7 ± 0.1
H2	46	*Erica* spp.	0.7 ± 0.0	150 ± 0	16.0 ± 0.0	2.4 ± 0.6	4.2 ± 0.0	27.7 ± 0.4	45.3 ± 0.2	11.2 ± 2.0	2.1 ± 0.4
H3	42	Multifloral*[2]	0.6 ± 0.0	138 ± 1	16.1 ± 0.1	35.0 ± 6.4	4.0 ± 0.1	23.6 ± 0.3	39.4 ± 0.4	9.5 ± 0.3	1.4 ± 0.2
H4	52	*Erica* spp.	0.5 ± 0.0	150 ± 0	16.2 ± 0.0	44.9 ± 8.8	3.9 ± 0.0	23.5 ± 0.2	41.2 ± 0.3	9.9 ± 1.0	0.7 ± 0.1
H5	19	Multifloral	0.5 ± 0.0	150 ± 0	16.8 ± 0.0	43.3 ± 3.7	3.9 ± 0.0	27.1 ± 0.0	47.3 ± 0.1	9.8 ± 0.6	1.3 ± 0.3
H6	8%	Multifloral	0.5 ± 0.0	150 ± 0	16.1 ± 0.0	21.1 ± 11.0	4.3 ± 0.0	22.8 ± 1.5	38.3 ± 1.5	9.1 ± 0.5	1.1 ± 0.1
Incense											
I1	51	*P. undulatum*	0.2 ± 0.0	45 ± 1	15.7 ± 0.0	20.6 ± 1.3	3.7 ± 0.0	7.5 ± 0.1	15.8 ± 0.2	1.3 ± 0.2	0.4 ± 0.0
I2	47	*P. undulatum*	0.3 ± 0.0	80 ± 1	15.8 ± 0.0	83.1 ± 8.5	3.6 ± 0.1	11.6 ± 0.2	23.7 ± 0.5	1.1 ± 0.2	0.5 ± 0.1
I3	34	*P. undulatum*	0.2 ± 0.0	40 ± 5	15.0 ± 0.0	31.8 ± 0.3	3.9 ± 0.0	8.5 ± 0.1	17.2 ± 0.1	4.3 ± 0.2	0.2 ± 0.1
I4	40	*P. undulatum*	0.2 ± 0.0	71 ± 2	15.9 ± 0.0	31.9 ± 2.6	4.0 ± 0.0	7.8 ± 0.2	18.1 ± 0.3	1.5 ± 0.7	0.4 ± 0.1
Lavender											
L1	43	*Lavender* spp.	0.1 ± 0.0	32 ± 1	15.5 ± 0.0	20.9 ± 0.3	3.6 ± 0.0	14.1 ± 0.0	27.8 ± 0.2	5.2 ± 1.0	0.7 ± 0.0
L2	12	Multifloral*[3]	0.2 ± 0.0	32 ± 1	15.3 ± 0.0	12.5 ± 1.0	3.5 ± 0.1	14.8 ± 0.4	26.3 ± 0.5	11.2 ± 0.7	0.6 ± 0.1
L3	36	*Lavender* spp.	0.1 ± 0.0	30 ± 1	15.7 ± 0.0	8.1 ± 2.5	3.4 ± 0.0	13.8 ± 0.2	26.8 ± 0.2	5.1 ± 0.9	0.5 ± 0.1
L4	14	Multifloral*[3]	0.1 ± 0.0	54 ± 2	14.2 ± 0.0	48.3 ± 1.3	3.4 ± 0.1	10.2 ± 0.3	21.8 ± 0.3	2.9 ± 0.7	0.4 ± 0.0
L5	35	*Lavender* spp.	0.2 ± 0.0	66 ± 3	14.8 ± 0.0	67.0 ± 5.3	3.8 ± 0.0	14.7 ± 0.2	28.7 ± 0.1	1.4 ± 0.3	0.6 ± 0.1
L6	8	Multifloral	0.1 ± 0.0	42 ± 3	14.2 ± 0.0	19.1 ± 6.9	3.6 ± 0.0	9.4 ± 0.4	20.7 ± 0.8	2.5 ± 0.0	0.5 ± 0.1
L7	5	Multifloral	0.1 ± 0.0	34 ± 1	15.8 ± 0.01	15.4 ± 0.6	3.6 ± 0.1	12.7 ± 0.2	24.8 ± 0.2	4.7 ± 0.5	0.5 ± 0.1
L8	4	*Echium* spp.	0.1 ± 0.0	26 ± 1	15.3 ± 0.0	8.6 ± 0.1	3.2 ± 0.0	11.8 ± 0.1	21.8 ± 0.3	8.4 ± 0.3	0.4 ± 0.0
Orange											
O1	3	Multifloral	0.2 ± 0.0	72 ± 0	15.0 ± 0.0	61.1 ± 12.5	3.5 ± 0.0	17.1 ± 0.4	34.4 ± 0.2	2.6 ± 0.2	0.5 ± 0.1
O2	23	*Citrus* spp.	0.1 ± 0.0	43 ± 1	15.8 ± 0.0	35.1 ± 4.5	3.7 ± 0.0	12.2 ± 0.0	23.3 ± 0.2	3.3 ± 0.2	0.4 ± 0.0
O3	1	Multifloral*[4]	0.1 ± 0.0	48 ± 1	16.0 ± 0.0	15.4 ± 3.0	3.4 ± 0.2	14.6 ± 0.2	26.8 ± 0.3	8.6 ± 0.1	0.6 ± 0.1
O4	54	*Citrus* spp.	0.2 ± 0.0	78 ± 1	15.1 ± 0.0	22.0 ± 4.6	3.6 ± 0.0	14.3 ± 0.5	28.9 ± 1.0	3.4 ± 0.1	0.4 ± 0.2
O5	81	*Citrus* spp.	0.2 ± 0.0	42 ± 1	15.1 ± 0.0	26.4 ± 2.0	3.6 ± 0.1	13.6 ± 0.6	28.8 ± 0.5	3.0 ± 0.2	0.4 ± 0.0
O6	8	Multifloral	0.3 ± 0.0	63 ± 2	16.5 ± 0.0	60.2 ± 4.3	3.3 ± 0.0	26.0 ± 0.0	48.1 ± 0.0	5.1 ± 0.5	0.6 ± 0.2

(*continued on next page*)

Table 2 (*continued*)

AH	PA (%)	Botanical Source	Electrical conductivity (mS/cm)	Colour (mm Pfund)	Moisture (%)	HMF (mg/kg)	pH	Free acidity (meq/kg)	Total acidity (meq/kg)	DA (schade number)	Proline (mg/g)
O7	57	*Citrus* spp.	0.1 ± 0.0	31 ± 1	15.9 ± 0.0	26.9 ± 1.3	3.6 ± 0.0	12.2 ± 0.1	24.3 ± 0.4	4.7 ± 0.1	0.6 ± 0.1
O8	15	*Citrus* spp.	0.2 ± 0.0	42 ± 2	16.0 ± 0.0	18.8 ± 0.4	3.5 ± 0.0	15.7 ± 0.1	29.2 ± 0.1	8.9 ± 0.4	0.2 ± 0.0
O9	35	*Citrus* spp.	0.1 ± 0.0	36 ± 2	14.5 ± 0.0	38.0 ± 1.5	3.7 ± 0.0	13.3 ± 0.0	25.9 ± 0.1	3.0 ± 0.1	0.4 ± 0.1
Rape											
R1	28	Multifloral	0.1 ± 0.0	70 ± 1	17.4 ± 0.0	21.1 ± 0.9	3.5 ± 0.0	10.4 ± 0.1	21.0 ± 0.1	13.8 ± 2.0	0.3 ± 0.0
R2	45	Multifloral*[5]	0.1 ± 0.0	37 ± 3	18.6 ± 0.0	22.2 ± 0.2	3.2 ± 0.0	14.8 ± 0.1	27.2 ± 0.1	15.8 ± 0.5	0.4 ± 0.0
Raspberry											
Rb1	19	Multifloral	0.2 ± 0.0	32 ± 1	16.6 ± 0.0	15.2 ± 0.3	3.6 ± 0.0	13.9 ± 0.1	24.0 ± 0.1	10.9 ± 2.3	0.5 ± 0.0
Rb2	53	*Rubus* spp.	0.3 ± 0.0	33 ± 1	16.9 ± 0.0	28.6 ± 0.3	3.3 ± 0.0	24.7 ± 0.1	45.8 ± 0.3	15.6 ± 0.3	0.9 ± 0.1
Rosemary											
Ros1	1	Multifloral	0.5 ± 0.0	84 ± 0	16.1 ± 0.0	4.6 ± 4.2	4.1 ± 0.0	23.5 ± 0.2	35.0 ± 0.3	19.2 ± 0.5	1.3 ± 0.5
Sunflower											
Sf1	13	*H. annuus*	0.3 ± 0.0	66 ± 1	16.1 ± 0.0	22.5 ± 3.4	3.4 ± 0.1	18.8 ± 0.2	35.0 ± 0.8	15.1 ± 0.7	0.9 ± 0.2
Sf2	15	*H. annuus*	0.4 ± 0.0	80 ± 2	16.4 ± 0.0	17.1 ± 3.0	3.6 ± 0.0	21.4 ± 0.1	38.6 ± 0.2	15.4 ± 0.4	1.3 ± 0.3
Sf3	58	*H. annuus*	0.3 ± 0.0	60 ± 2	15.5 ± 0.0	7.9 ± 4.9	3.6 ± 0.0	17.6 ± 0.3	32.3 ± 0.3	18.0 ± 0.8	1.0 ± 0.1
Strawberry tree											
St1	29	*A. unedo*	0.6 ± 0.0	150 ± 0	15.8 ± 0.0	29.1 ± 0.4	4.2 ± 0.0	14.7 ± 0.1	34.9 ± 0.3	0.1 ± 0.1	0.2 ± 0.0
St2	13	*A. unedo*	0.7 ± 0.0	115 ± 4	18.0 ± 0.0	31.2 ± 9.7	4.4 ± 0.0	17.5 ± 0.2	33.2 ± 0.4	0.2 ± 0.1	0.6 ± 0.0
St3	5	Multifloral	0.7 ± 0.0	101 ± 2	17.3 ± 0.0	28.8 ± 5.9	4.4 ± 0.0	19.3 ± 0.2	40.0 ± 0.2	4.4 ± 0.2	0.7 ± 0.1
St4	20	*A. unedo*	0.6 ± 0.0	107 ± 3	16.9 ± 0.0	2.8 ± 0.6	4.1 ± 0.1	22.5 ± 0.2	42.0 ± 0.2	10.7 ± 0.5	1.6 ± 0.0

AH: Analyzed honeys. PA: Pollen analysis. Each value is the mean ± standard deviation, n = 3. DA: Diastase activity. Ct: Carob tree. C: Chestnut. E: Eucalyptus. H: Bell heather. I: Incense. L: Lavender. O: Orange. R: Rape. Rb: Raspberry. Ros: Rosemary. Sf: Sunflower. St: Strawberry tree. *[1]: Predominance of *Eucalyptus* spp. pollen. *[2]: Predominance of *Erica* spp. pollen. *[3]: Predominance of *Lavandula* spp. pollen. *[4]: Predominance of *Echium* spp. pollen *[5]: Predominance of *Brassica* spp. pollen.

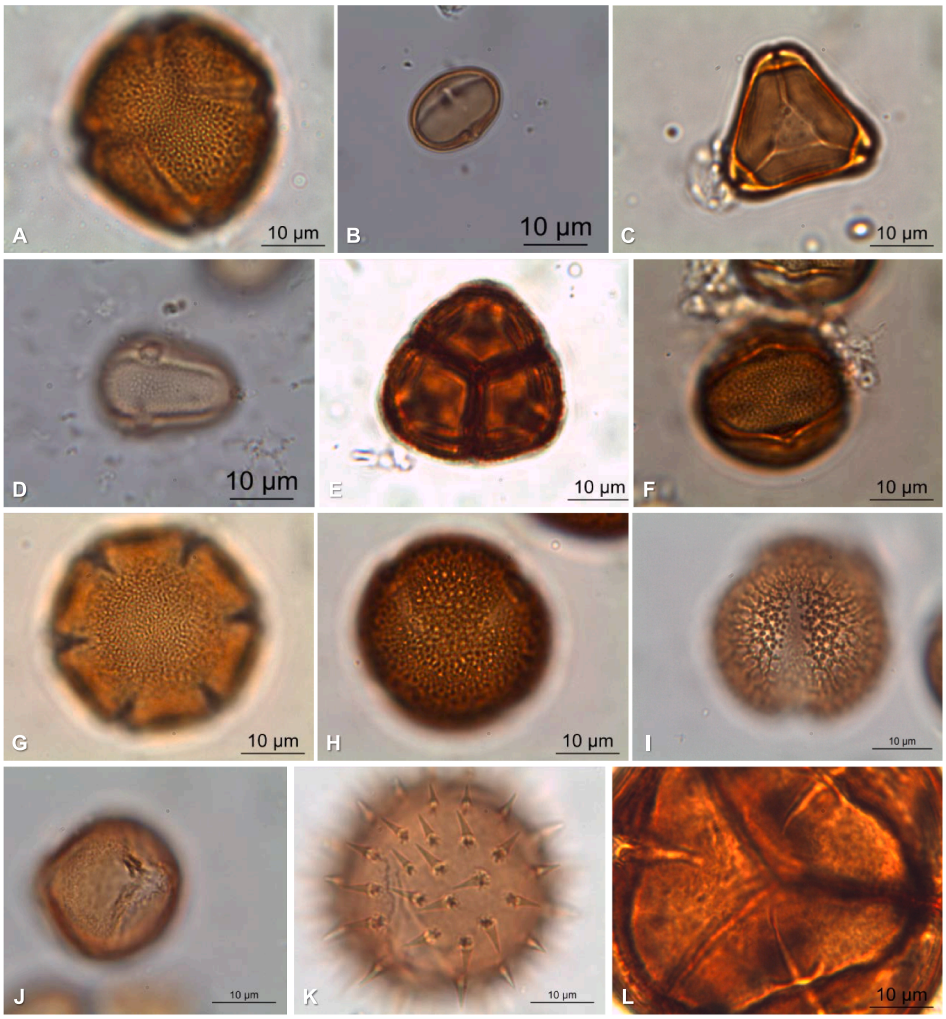


Fig. 2. Representative botanical species pollen types present in honeys of carob tree **A.** *Ceratonia siliqua* (polar view), chestnut **B.** *Castanea sativa* (equatorial view), eucalyptus **C.** *Eucalyptus* spp. (polar view), viper's bugloss **D.** *Echium* spp. (equatorial view), bell heather **E.** *Erica* spp. (polar view), incense **F.** *Pittosporum undulatum* (equatorial view), Lavender **G.** *Lavandula* spp. (polar view), orange **H.** *Citrus* spp. (polar view), rape **I.** *Brassica* spp. (polar view), raspberry **J.** *Rubus* spp. (polar view), sunflower **K.** *Helianthus annuus* (polar view), and strawberry tree **L.** *Arbutus unedo* (polar view). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2004). In the four studied honeys, *Arbutus unedo* pollen varied between 5 and 29%.

Although not labelled as viper's bugloss honey, the honeys labelled as lavender (L8), orange (O3) and rosemary (Ros1) honeys showed a percentage of *Echium* spp. pollen above 45%. Arroyo et al. (2017) suggested a minimum of 70% of *Echium* spp. pollen to consider this honey monofloral, thereby only L8 sample with 83% of this pollen type fitted this classification. Sample O3 was considered as multifloral with predominance of *Echium* spp. pollen type (61%), and Ros1 was classified as multifloral with 49% of this pollen.

3.1.1. Honey's classification regarding pollen type

As stated above, only some honeys confirmed monofloral labelling, after melissopalynological analysis. Overall these were grouped as follows: a) twenty seven confirmed monofloral label (53% of total honeys), specifically three of carob tree (Ct1, Ct3, Ct4), one of chestnut (C2), one of eucalyptus (E3), two of bell heather (H2, H4), four of incense (I1-I4), three of lavender (L1, L3, L5), six of orange (O2, O4, O5, O7, O8, O9), one of raspberry (Rb2), three of sunflower (Sf1-Sf3) and three of strawberry tree (St1, St2, St4), b) fifteen honeys were classified as multifloral label (29% of total honeys), namely, Ct2, Ct5, C1, E4, E5, H5, H6, L6, L7, O1, O6, R1, Rb1, Ros1, St3, b1) eight honeys were considered multifloral with predominance of a specific pollen type (16% of total honeys), specifically two with *Eucalyptus* spp. pollen (E1, E2), two with *Erica* spp. pollen (H1, H3), two with *Lavandula* spp. pollen (L2, L4), one with *Echium* spp. pollen (O3) and one with *Brassica* spp. pollen (R2), and c) L8 sample was reassigned as monofloral (2% of total honeys), being classified as viper's bugloss (*Echium* spp.) honey.

3.2. Microbiological quality

The studied honeys showed microbiological quality within the acceptance criteria for aerobic mesophilic bacteria, $<10^3$ CFU/g, and also for yeasts and moulds, $<10^2$ CFU/g. Low values of these microorganisms are indicative of an appropriate management of apiaries. These results are similar to those reported by Estevinho et al. (2012) for Portuguese honey collected in Trás-os-Montes region. Enterobacteriaceae were absent in all honeys (<10 CFU/g) including *E. coli* isolates after inoculation into enrichment broth medium. The absence of faecal coliforms are indicators of good sanitary quality. In this study one honey sample was contaminated with the sulphite-reducing *Clostridium* spp. The detection of sporulate bacteria in honey suggests contamination or environmental pollution, as these bacteria are ubiquitous in the farming environment, being the soil the main responsible in addition to dust, equipment and buildings. The presence of spores of this Gram positive bacteria in honey is the responsible for infant botulism (Finola et al., 2007). European Union legislation still lacks specifications concerning microbial contamination or hygiene of honey, being urgent to establish honey microbiological quality standards. In general, the analyzed honeys in this work showed very good microbiological quality, revealing to be safe products for human consumption.

Table 3
Main mono-, di- and trisaccharides (%) of monofloral and multifloral honeys or with predominance of a specific pollen type according to botanical source after melissopalynological analysis.

AH	FT	GL	ISM	MTS	MTL	MBS	SCR	TRL	TRN	ERL	MZT	RFS	FT + GL
Carob tree													
Ct1	39.5 ± 0.3	21.6 ± 0.8	1.4 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	n.d.	0.9 ± 0.3	2.0 ± 0.2	1.4 ± 0.5	n.d.	n.d.	n.d.	61.1
Ct2	36.1 ± 1.7	25.4 ± 1.4	0.7 ± 0.4	1.5 ± 0.6	0.2 ± 0.1	n.d.	n.d.	1.2 ± 0.5	1.8 ± 0.2	n.d.	n.d.	n.d.	61.5
Ct3	41.8 ± 8.4	33.3 ± 6.8	2.5 ± 0.9	1.0 ± 0.2	2.6 ± 0.6	n.d.	n.d.	0.6 ± 0.1	1.9 ± 0.5	n.d.	3.8 ± 0.2	n.d.	75.1
Ct4	42.8 ± 6.6	35.2 ± 5.7	1.9 ± 0.2	0.4 ± 0.0	1.9 ± 0.4	n.d.	n.d.	1.0 ± 0.0	1.6 ± 0.1	n.d.	n.d.	n.d.	78.0
Ct5	45.7 ± 4.7	22.9 ± 2.5	n.d.	0.9 ± 0.5	1.0 ± 0.3	n.d.	0.8 ± 0.1	1.6 ± 0.6	1.9 ± 0.2	n.d.	n.d.	n.d.	68.6
Chestnut													
C1	37.6 ± 5.0	29.6 ± 4.0	3.1 ± 0.5	3.9 ± 0.5	n.d.	n.d.	n.d.	1.4 ± 0.0	2.0 ± 0.3	n.d.	n.d.	n.d.	67.2
C2	47.5 ± 8.5	21.4 ± 1.9	6.7 ± 0.5	n.d.	5.2 ± 0.7	n.d.	n.d.	n.d.	1.9 ± 0.4	n.d.	n.d.	n.d.	69.0
Eucalyptus													
E1*[1]	48.8 ± 7.1	21.1 ± 2.9	6.1 ± 0.3	n.d.	5.0 ± 0.2	n.d.	n.d.	1.1 ± 0.3	2.4 ± 0.2	n.d.	n.d.	n.d.	69.1
E2*[1]	50.7 ± 5.1	22.4 ± 2.0	4.7 ± 0.4	n.d.	4.4 ± 0.5	n.d.	n.d.	2.5 ± 0.1	1.9 ± 0.2	n.d.	n.d.	n.d.	73.1
E3	37.2 ± 5.0	26.7 ± 3.4	3.4 ± 1.2	n.d.	3.5 ± 0.9	n.d.	n.d.	0.5 ± 0.2	1.8 ± 0.3	n.d.	n.d.	n.d.	63.9
E4	37.0 ± 0.9	29.9 ± 0.7	0.9 ± 0.2	3.2 ± 0.1	2.1 ± 0.1	n.d.	n.d.	1.7 ± 0.1	1.8 ± 0.1	n.d.	n.d.	n.d.	66.9
E5	47.3 ± 1.6	27.7 ± 0.7	1.8 ± 0.2	1.2 ± 0.1	1.5 ± 0.1	n.d.	n.d.	1.0 ± 0.0	1.3 ± 0.1	0.2 ± 0.1	n.d.	n.d.	75.0
Bell heather													
H1*[2]	40.2 ± 3.5	30.1 ± 2.9	0.8 ± 0.1	0.4 ± 0.5	1.2 ± 0.3	n.d.	n.d.	1.0 ± 0.2	1.4 ± 0.0	n.d.	n.d.	n.d.	70.3
H2	35.0 ± 0.5	27.8 ± 0.3	2.3 ± 0.5	2.1 ± 0.7	4.4 ± 1.4	n.d.	n.d.	1.4 ± 0.0	2.1 ± 0.4	n.d.	n.d.	n.d.	62.8
H3*[2]	35.8 ± 1.1	28.2 ± 0.9	1.6 ± 0.2	1.6 ± 0.1	2.9 ± 0.6	n.d.	n.d.	1.3 ± 0.0	1.7 ± 0.3	n.d.	n.d.	n.d.	64.0
H4	40.1 ± 8.5	22.4 ± 4.9	2.2 ± 1.4	3.1 ± 1.9	6.8 ± 2.7	n.d.	n.d.	2.2 ± 0.5	3.1 ± 1.1	n.d.	n.d.	n.d.	62.5
H5	36.7 ± 4.6	25.4 ± 3.2	2.1 ± 0.6	6.5 ± 1.4	3.7 ± 0.3	n.d.	n.d.	2.7 ± 0.4	2.3 ± 0.5	n.d.	n.d.	n.d.	62.1
H6	42.0 ± 1.4	26.2 ± 7.8	2.5 ± 1.0	6.2 ± 2.5	2.8 ± 1.6	n.d.	n.d.	2.1 ± 0.7	2.9 ± 1.1	n.d.	n.d.	n.d.	68.2
Incense													
I1	32.4 ± 1.2	30.5 ± 1.8	0.7 ± 0.4	0.8 ± 0.6	0.9 ± 0.5	n.d.	0.3 ± 0.0	0.6 ± 0.1	1.1 ± 0.1	n.d.	n.d.	n.d.	62.9
I2	33.0 ± 3.2	29.2 ± 3.0	0.9 ± 0.1	1.5 ± 0.0	1.1 ± 0.1	n.d.	0.3 ± 0.0	0.6 ± 0.0	1.5 ± 0.2	n.d.	n.d.	n.d.	62.2
I3	43.3 ± 7.4	27.6 ± 0.4	n.d.	0.9 ± 0.7	1.1 ± 0.5	n.d.	0.7 ± 0.1	0.4 ± 0.2	1.9 ± 0.1	1.0 ± 0.6	n.d.	n.d.	70.9
I4	40.0 ± 1.1	26.7 ± 1.5	0.2 ± 0.3	0.9 ± 0.2	0.5 ± 0.2	n.d.	0.6 ± 0.1	0.3 ± 0.2	1.0 ± 0.2	n.d.	n.d.	n.d.	66.7
Lavender													
L1	40.8 ± 8.6	34.9 ± 7.3	1.9 ± 0.9	0.6 ± 0.1	3.2 ± 0.7	n.d.	0.9 ± 0.3	0.3 ± 0.1	1.7 ± 0.4	1.8 ± 0.4	n.d.	n.d.	75.7
L2*[3]	44.8 ± 8.4	28.3 ± 2.0	1.9 ± 0.2	1.0 ± 0.2	2.6 ± 0.2	n.d.	0.2 ± 0.0	0.6 ± 0.0	2.0 ± 0.2	n.d.	n.d.	n.d.	73.1
L3	34.7 ± 3.5	34.4 ± 3.3	2.2 ± 0.3	0.7 ± 0.5	3.0 ± 0.4	n.d.	0.5 ± 0.0	0.5 ± 0.1	1.5 ± 0.1	1.3 ± 0.2	n.d.	n.d.	69.1
L4*[3]	40.1 ± 6.0	24.5 ± 0.9	1.1 ± 0.5	3.2 ± 0.2	2.0 ± 0.1	n.d.	n.d.	0.5 ± 0.2	0.4 ± 0.0	n.d.	n.d.	n.d.	64.6
L5	37.9 ± 1.3	25.4 ± 0.7	n.d.	0.7 ± 0.1	1.3 ± 0.2	n.d.	n.d.	1.0 ± 0.1	2.0 ± 0.1	n.d.	1.3 ± 0.4	n.d.	63.3
L6	42.9 ± 0.4	23.6 ± 0.8	2.5 ± 0.3	4.2 ± 0.2	1.8 ± 0.1	n.d.	n.d.	0.8 ± 0.1	2.5 ± 0.1	1.6 ± 0.2	n.d.	n.d.	66.5
L7	34.5 ± 6.4	29.4 ± 5.6	n.d.	1.3 ± 0.2	1.0 ± 0.2	n.d.	n.d.	0.7 ± 0.1	1.4 ± 0.4	1.8 ± 0.4	n.d.	n.d.	63.9
L8	40.9 ± 0.1	26.3 ± 0.6	0.5 ± 0.5	0.7 ± 0.5	0.7 ± 0.1	n.d.	0.1 ± 0.0	0.5 ± 0.2	0.9 ± 0.2	0.9 ± 0.0	n.d.	n.d.	67.2
Orange													
O1	38.0 ± 1.4	26.5 ± 1.0	1.7 ± 0.4	2.8 ± 0.0	1.3 ± 0.1	n.d.	n.d.	1.2 ± 0.4	2.5 ± 0.3	n.d.	n.d.	n.d.	64.5
O2	41.0 ± 1.2	30.3 ± 1.2	n.d.	2.0 ± 2.2	1.7 ± 1.4	2.5 ± 1.4	3.1 ± 2.7	0.9 ± 0.8	2.4 ± 0.8	n.d.	n.d.	n.d.	71.3
O3*[4]	34.2 ± 3.0	26.9 ± 2.3	1.1 ± 0.1	0.7 ± 0.3	0.8 ± 0.2	n.d.	0.3 ± 0.1	0.9 ± 0.0	1.7 ± 0.2	2.2 ± 0.4	n.d.	n.d.	61.1
O4	37.3 ± 0.0	28.8 ± 0.2	0.9 ± 0.0	1.7 ± 0.1	1.4 ± 0.1	n.d.	0.7 ± 0.0	0.9 ± 0.1	1.8 ± 0.2	n.d.	n.d.	n.d.	66.1
O5	39.4 ± 2.0	31.6 ± 1.5	n.d.	1.3 ± 0.4	0.8 ± 0.2	n.d.	0.5 ± 0.1	0.8 ± 0.1	1.4 ± 0.1	n.d.	n.d.	n.d.	71.0
O6	35.4 ± 2.2	28.8 ± 1.6	n.d.	0.4 ± 0.2	0.8 ± 0.4	n.d.	n.d.	0.6 ± 0.1	1.5 ± 0.5	n.d.	n.d.	n.d.	64.2
O7	41.7 ± 0.9	31.7 ± 0.6	n.d.	1.0 ± 0.3	1.0 ± 0.3	n.d.	2.1 ± 0.1	0.9 ± 0.1	2.0 ± 0.1	n.d.	n.d.	n.d.	73.4
O8	40.2 ± 1.1	32.5 ± 0.9	0.8 ± 0.1	n.d.	1.4 ± 0.2	n.d.	n.d.	0.7 ± 0.1	2.2 ± 0.1	n.d.	n.d.	n.d.	72.7
O9	34.3 ± 1.6	27.3 ± 1.3	n.d.	0.7 ± 0.6	1.2 ± 0.4	n.d.	0.7 ± 0.2	1.0 ± 0.3	1.3 ± 0.6	n.d.	n.d.	n.d.	61.6
Rape													
R1	34.8 ± 1.0	32.8 ± 0.9	1.5 ± 0.1	1.7 ± 0.2	n.d.	n.d.	n.d.	0.6 ± 0.1	1.5 ± 0.0	n.d.	n.d.	n.d.	67.6
R2*[5]	38.9 ± 3.7	27.7 ± 2.5	n.d.	0.4 ± 0.5	1.1 ± 0.7	n.d.	0.5 ± 0.5	0.3 ± 0.2	1.6 ± 0.3	n.d.	n.d.	n.d.	66.6
Raspberry													
Rb1	39.6 ± 4.9	31.8 ± 4.2	0.4 ± 0.2	0.7 ± 0.1	0.3 ± 0.1	n.d.	n.d.	1.1 ± 0.1	1.7 ± 0.1	n.d.	n.d.	n.d.	71.4
Rb2	39.3 ± 0.6	31.9 ± 0.4	0.2 ± 0.2	1.2 ± 0.3	0.6 ± 0.1	n.d.	0.2 ± 0.0	0.6 ± 0.0	0.8 ± 0.1	n.d.	n.d.	n.d.	71.2
Rosemary													
Ros1	33.9 ± 1.0	28.3 ± 1.9	2.0 ± 0.3	2.4 ± 0.3	1.2 ± 0.2	n.d.	n.d.	0.8 ± 0.1	1.8 ± 0.3	n.d.	n.d.	n.d.	62.2
Sunflower													
Sf1	41.3 ± 1.2	35.5 ± 1.0	0.6 ± 0.8	0.6 ± 0.5	0.9 ± 0.3	n.d.	n.d.	0.6 ± 0.2	1.0 ± 0.1	n.d.	n.d.	n.d.	76.8
Sf2	38.2 ± 0.9	32.6 ± 1.0	0.4 ± 0.7	1.0 ± 0.7	0.6 ± 0.3	n.d.	n.d.	0.7 ± 0.2	0.8 ± 0.0	n.d.	n.d.	n.d.	70.8

(*continued on next page*)

Table 3 (*continued*)

AH	FT	GL	ISM	MTS	MTL	MBS	SCR	TRL	TRN	ERL	MZT	RFS	FT + GL
Sf3	41.1 ± 5.0	29.4 ± 0.8	0.4 ± 0.5	0.6 ± 0.1	0.6 ± 0.2	n.d.	0.4 ± 0.1	0.5 ± 0.2	0.7 ± 0.1	n.d.	n.d.	n.d.	70.5
Strawberry tree													
St1	34.6 ± 2.3	30.8 ± 2.0	2.0 ± 1.3	n.d.	2.8 ± 1.0	n.d.	n.d.	0.9 ± 0.2	1.7 ± 0.5	0.6 ± 0.3	n.d.	n.d.	65.4
St2	38.8 ± 1.3	22.9 ± 0.6	n.d.	0.6 ± 0.4	0.7 ± 0.3	n.d.	1.6 ± 0.2	0.7 ± 0.3	1.2 ± 0.1	n.d.	n.d.	n.d.	61.7
St3	39.9 ± 1.5	25.5 ± 1.1	1.1 ± 0.5	2.7 ± 0.5	0.8 ± 0.6	n.d.	0.3 ± 0.1	0.6 ± 0.0	1.9 ± 0.2	n.d.	n.d.	n.d.	65.4
St4	39.4 ± 0.4	33.0 ± 0.5	2.7 ± 0.1	1.0 ± 0.0	3.2 ± 0.1	n.d.	n.d.	1.0 ± 0.1	2.0 ± 0.1	n.d.	n.d.	n.d.	72.4

AH: Analyzed honeys. FT: Fructose. GL: Glucose. ISM: Isomaltose. MTS: Maltose. MTL: Maltulose. MBS: Melibiose. SCR: Sucrose. TRL: Trehalose. TRN: Turanose. ERL: Erlose. MZT: Melezitose. RFS: Raffinose. Each value is the mean ± standard deviation, n = 3. Ct: Carob tree. C: Chestnut. E: Eucalyptus. H: Bell heather. I: Incense. L: "Lavender. O: Orange. R: Rape. Rb: Raspberry. Ros: Rosemary. Sf: Sunflower. St: Strawberry tree. n.d.: not detected. FT + GL: Fructose + Glucose. *[1] Predominance of *Eucalyptus* spp. pollen. *[2] Predominance of *Erica* spp. pollen. *[3] Predominance of *Lavender* spp. pollen. *[4] Predominance of *Echium* spp. pollen. *[5] Predominance of *Brassica* spp. Pollen.

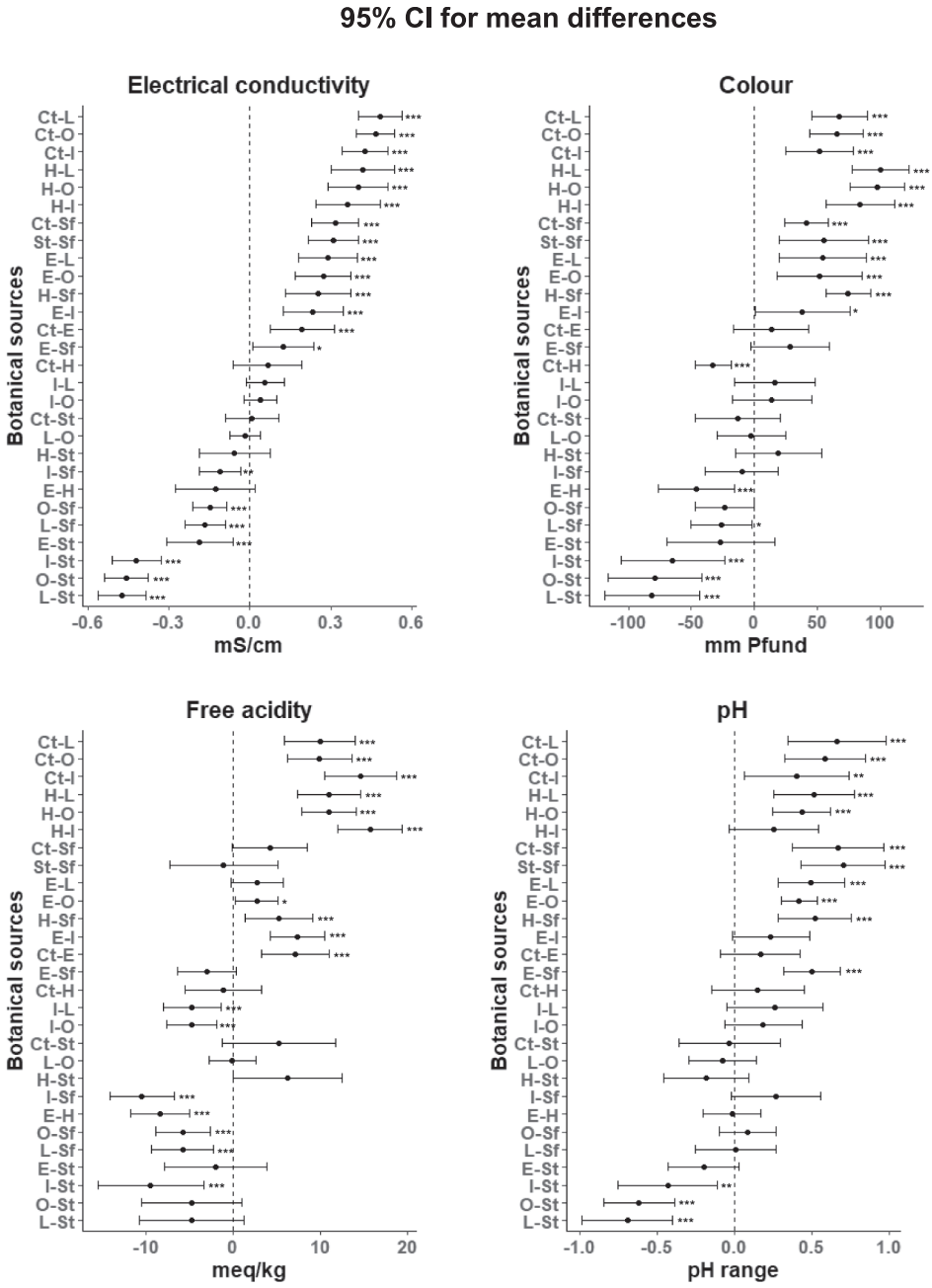


Fig. 3. Mean differences between pairs of monofloral honeys, for electrical conductivity, colour, free acidity, and pH parameters. *yy* axis represent pairs of monofloral honeys and *xx* axis represent the mean differences of the analyzed parameter and the corresponding 95% CI (Confidence interval). Ct: Carob tree, E: Eucalyptus, H: Bell heather, I: Incense, L: Lavender, O: Orange, Sf: Sunflower, St: Strawberry tree. Statistical significance is indicated for each pair: ***: $p \leq 0.001$, **: $p \leq 0.01$, *: $p \leq 0.05$. The dotted vertical line at x = 0 corresponds to no difference in mean between the elements of the pair. For each pair (represented at the *yy* axis), the dot corresponds to the difference of the means and the horizontal segments represent the CI for the mean difference. Pairs for which the CI segment crosses the dotted line are not statistically different in mean. Those with the CI segment on the right side of the dotted line are the ones for which the first element of the pair has a significantly higher mean. The opposite occurs for the pairs with the CI segments on the left of the dotted line.

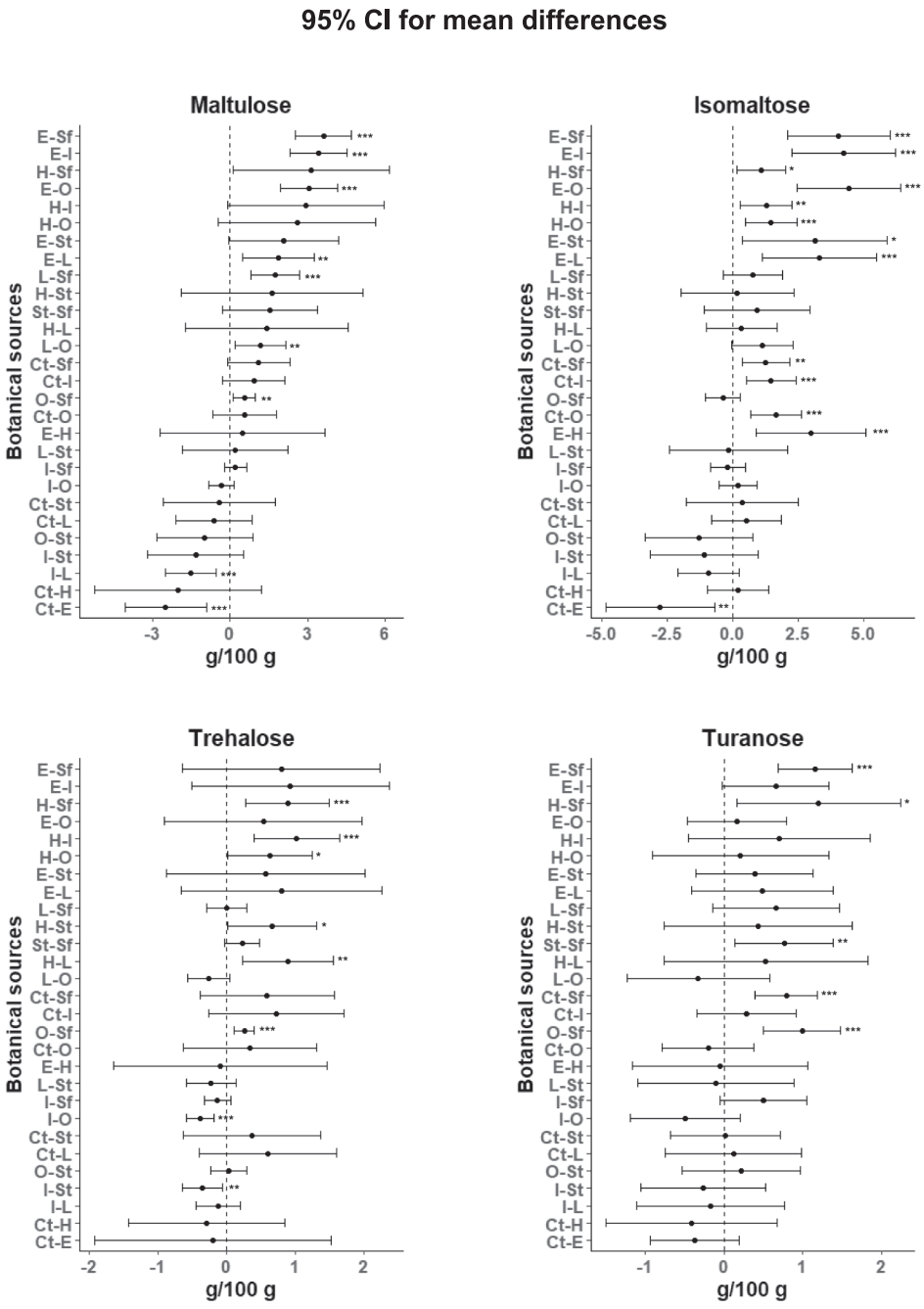


Fig. 4. Mean differences between pairs of monofloral honeys, for maltulose, isomaltose, trehalose and turanose. *yy* axis represent pairs of monofloral honeys and *xx* axis represent the mean differences of the analyzed parameter and the corresponding 95% CI (Confidence interval). Ct: Carob tree, E: Eucalyptus, H: Bell heather, I: Incense, L: Lavender, O: Orange, Sf: Sunflower, St: Strawberry tree. Statistical significance is indicated for each pair: ***: $p \leq 0.001$, **: $p \leq 0.01$, *: $p \leq 0.05$. The dotted vertical line at x = 0 corresponds to no difference in mean between the elements of the pair. For each pair (represented at the *yy* axis), the dot corresponds to the difference of the means and the horizontal segments represent the CI for the mean difference. Pairs for which the CI segment crosses the dotted line are not statistically different in mean. Those with the CI segment on the right side of the dotted line are the ones for which the first element of the pair has a significantly higher mean. The opposite occurs for the pairs with the CI segments on the left of the dotted line.

3.3. Physicochemical characterization of honeys

The obtained average data for colour index, moisture, electrical conductivity, HMF, pH, free acidity, total acidity, diastase activity and proline, for all honeys, is detailed in Table 2, while Table 3 shows the average values for the sugar profile.

To assess generalized linear regression models that could predict the effects of the botanical source on the physicochemical parameters, only the honeys confirmed as monofloral or those with a predominance of a specific pollen type (with a minimum number of three honeys per honey type), where selected for the application of generalized estimating equations (GEE). To identify significant differences ($p < 0.05$) amongst honeys from different botanical source, pairwise comparisons between honey types were also evaluated.

Using the analyzed physicochemical parameters, detailed in the following sections, and comparing pairs of monofloral honeys or with predominance of a specific pollen, it was possible to define graphs of degree of relatedness between honey types, [Figs. 3 and 4, and Supplementary File (S) Fig. S2 and S3]. For instance, for electrical conductivity, carob tree (Ct) and lavender (L) honeys (top left corner) showed significant differences ($p \leq 0.001$) whereas lavender (L) and orange (O) honeys did not show significant differences between them (Fig. 3). The dotted vertical line at x = 0 helps to understand the results. The honey pairs whose confidence interval (CI) line crosses the line are not significantly different in relation to the parameter analyzed. When the CI line for the mean difference of the honey pairs is on the right of the dotted line, it means that the first element of the pair has a significantly higher value than the other element, for the assessed physicochemical parameter. On the contrary, when the CI line for the mean difference of the honey pairs is on the left of the dotted line, it means that the second element of the pair has a significantly higher value than the first element, regarding the evaluated physicochemical parameter.

The results presented and discussed in the following sections, concern only the confirmed monofloral honeys and the multifloral ones with predominance of a specific pollen type. Whenever, for comparison purposes, multifloral honeys are addressed, this is specifically mentioned.

3.3.1. Electrical conductivity

The higher the concentration of mineral salts, organic acids, and proteins, the higher the corresponding conductivity. Mineral content depends on various factors such as botanical source, geographic origin or soil composition, and can be used for botanical source discrimination (Alves et al., 2013).

The values of electrical conductivity obtained in this study comply with the specified by Portuguese and European legislations for nectar honey (Decreto Lei no 214/2003; Council directive 2001/110/EC, 2002). Except for chestnut and one multifloral honey, all had electrical conductivity values less than the maximum limit (0.8 mS/cm). Chestnut honey C2 (0.81 mS/cm), and one multifloral C5 (1.14 mS/cm), showed values above the limit (Table 2). C2 chestnut honey value was similar to that reported by Rodríguez-Flores et al. (2019a) also for this honey type. However, chestnut honey from Greece showed an even higher value, 1.68 mS/cm (Rodopoulou et al., 2021). Electrical conductivity was significantly different ($p < 0.05$) between different pairs of honeys, Fig. 3.

Carob tree, bell heather and strawberry tree honeys showed high conductivity (0.5–0.7 mS/cm) although below the standard limit. Comparable results were found for Moroccan and Portuguese carob tree honeys (Terrab et al., 2003; Aazza et al., 2013). Other Portuguese bell heather honeys showed lower electrical conductivity values (Estevinho et al., 2012), although higher ones were obtained for Spanish honeys from Galicia (Rodríguez-Flores et al., 2019b).

Eucalyptus honeys showed electrical conductivity values (0.4–0.5 mS/cm) similar to those reported in Northwest Iberian Peninsula (Rodríguez-Flores et al., 2014), or Algerian eucalyptus honeys (Makhloufi et al., 2021).

On the other hand, incense, lavender, orange, rape, raspberry, viper's bugloss, and sunflower honeys presented lower values (0.1–0.4 mS/cm). Orange honeys analyzed in this work showed similar values (0.1–0.2 mS/cm) to those obtained by Aazza et al. (2013) and Alves et al. (2013) (0.2 mS/cm in both cases), also for Portuguese orange honeys. Nevertheless, these values were lower than those found for Algerian (Homrani et al., 2020) or Greek orange honeys (Rodopoulou et al., 2021) (0.3 mS/cm in both cases). Electrical conductivity values for sunflower honeys were also below those reported in Czech Republic, Republic of Serbia, and Spain (Juan-Borrás et al., 2014; Sakač et al., 2019). The results of incense honey comply with Commission Delegated Regulation (EU) PDO-PT-0268-AM01 (2019) for Azores honey. It is also worth mentioning that the lowest electrical conductivity values were found for lavender honeys (0.1–0.2 mS/cm), even lower than those reported by Anjos et al. (2018) for Portuguese lavender honey (0.2–0.4 mS/cm).

3.3.2. Colour index

Honey colour's varies from nearly colourless to dark brown (Bogdanov et al., 2002), and is mostly determined by the honey's botanical source, ash content, storage time and temperature, as well as by the presence of compounds, such as chlorophylls, carotenoids, flavonoids, tannins derivatives and polyphenols (da Silva et al., 2016; Pita-Calvo and Vázquez, 2017; Sakač et al., 2019).

The honeys showed a colour range from white to dark amber (Table 2, Fig. S1), with significant differences ($p < 0.05$) between most of them (Fig. 3), according to pairwise comparisons between honeys from different botanical sources. Lighter honeys included those from incense, lavender, orange, rape, raspberry, viper's bugloss, and sunflower whilst darker honeys were those from carob tree, chestnut, eucalyptus, bell heather, and strawberry tree (Fig. S1).

Carob tree honeys colour range (106–118 mm Pfund) was similar to Moroccan carob tree honeys (Terrab et al., 2003) although darker than Sicilian honeys (Ferrauto & Pavone, 2013).

The dark amber colour of C2 chestnut honey (147 mm Pfund), is in accordance to that reported for Spanish honeys from Galicia (Rodríguez-Flores et al., 2019a).

Eucalyptus honeys evidenced light amber and amber colour (75–113 mm Pfund) similar to values reported for Portuguese, Moroccan and Spanish honeys (Rodríguez-Flores et al., 2014; Karabagias et al., 2018; Bobis et al., 2020).

Bell heather honeys showed dark amber colour (>130 mm Pfund) typical of bell heather honey from *Erica* spp., in Portugal and Spain (Karabagias et al., 2018; Rodríguez-Flores et al., 2019b).

Incense honeys from the Azores islands, evidenced a variation in colour according to the island of origin, 40–45 mm Pfund in São Miguel Island, 71–80 mm Pfund in Pico Island (Table 2). Thus only São Miguel incense honeys meet EU colour specifications for Azores honey Commission Delegated Regulation (EU) (PDO-PT-0268-AM01, 2019), that should exceed 50 mm Pfund.

Lavender honeys showed a colour range of 30 to 66 mm Pfund. The lowest value was assigned to sample L8, which after pollen analysis indicated viper's bugloss honey as botanical source. The remaining lavender labelled honeys were lighter than other Portuguese lavender honeys from the north of Portugal (Gomes et al., 2011).

Orange honeys colour range (31–78 mm Pfund) was similar to the obtained for Algerian, Moroccan and Spanish honeys (Juan-Borrás et al., 2015; El Menyiy et al., 2020; Homrani et al., 2020), although higher than those of other Portuguese orange honeys (31 mm Pfund) (Aazza et al., 2013). Nevertheless, in the study of Aazza et al. (2013), there was no melissopalynological analysis, and the designations were only according to the label, with no guarantee that this was indeed the same honey type.

Honey sample R2 with predominance of *Brassica* spp. pollen revealed 37 mm Pfund, comparable to similar rape honeys from Romania (20–34 mm Pfund) (Pauliuc et al., 2020).

Raspberry honey sample Rb2 showed white colour (33 mm Pfund), different from that obtained for Spanish and Romanian honeys (Escuredo et al., 2012; Pauliuc et al., 2020), which evidenced amber and light amber colours, respectively.

Sunflower honeys colour range (60–80 mm) was similar to the obtained for Spanish, Romanian and Czech Republic honeys (Juan-Borrás et al., 2014), although different from those reported by Pauliuc et al. (2020) for Romanian honeys with a mean Pfund value of 38 mm.

Strawberry tree honeys showed amber and dark amber colour (107–150 mm Pfund). The darker ones were produced in Alentejo and in Algarve (Table 2), although slightly darker than those observed in strawberry tree honeys from the same geographical origin (Aazza et al., 2013).

3.3.3. Moisture content

The moisture level of the analysed honey samples ranged between 14 and 19% (Table 2). Pairwise comparisons between honey types showed that bell heather honeys exhibited significant differences ($p < 0.05$) with respect to incense and to lavender honeys (Fig. 3) and these last honey types were statistically different from orange honeys ($p < 0.05$). In all cases, this parameter complied with the international standards (Council directive 2001/110/EC, 2002), which defines 20% as the higher moisture limit for honeys placed on market. Besides, the values are also in agreement with previous studies on Portuguese chestnut, eucalyptus or bell heather honeys (Feás et al., 2010; Karabagias et al., 2018). It is worth mentioning that the legislation for Azores honey (Commission Delegated Regulation (EU) PDO-PT-0268-AM01, 2019) recommends 18% as the maximum allowed, which was fulfilled considering the moisture content between 15 and 16% for the incense honey samples.

3.3.4. Hydroxymethylfurfural (HMF)

In the present study eight honeys, of which, three monofloral (bell heather H4, incense I2, lavender L5), one multifloral with predominance of *Lavandula* spp. pollen (lavender L4), and four multifloral (eucalyptus E5, bell heather H5 orange O1 and O6), did not comply with the Council directive 2001/110/EC (2002) requirements for honey, which establish the HMF limit of 40 mg / kg. Of these honeys, six of them (H4, H5, I2, L4, L5, O1) were produced in 2015/2016, meaning the HMF values obtained could be explained by the aging of the honeys. Pairwise comparisons between honey types, indicated that only carob tree and sunflower honeys showed significantly differences between them ($p < 0.05$) (Fig. S2).

3.3.5. pH

The pH values ranged from 3.2 to 4.4, with pairwise comparisons showing significant differences ($p < 0.05$) between honey samples from different botanical sources (Fig. 3). The values obtained were in accordance with the expected range for nectar honey (3.2 to 4.5), which, together with its natural acidity, inhibit the growth of microorganisms (da Silva et al., 2016). The low pH values of honey are associated with the presence of organic acids, mainly gluconic acid, and inorganic ions such as phosphate and chloride (Pita-Calvo and Vázquez, 2017).

Carob tree, chestnut and strawberry tree honeys evidenced higher pH values (4.1–4.4) whilst lavender, orange, rape, raspberry, viper's-bugloss, and sunflower honeys showed lower values (3.2–3.8). The results are similar to those reported for Portuguese chestnut, eucalyptus, bell heather or strawberry tree honeys (Aazza et al., 2013; Alves et al., 2013; Karabagias et al., 2018).

3.3.6. Acidity

Free acidity is a parameter characterized by the presence of organic acids in equilibrium with their corresponding lactones and some inorganic ions, such as phosphate, sulphate and chloride (Alves et al., 2013; Pita-Calvo and Vázquez, 2017). Like pH, free and lactonic acidity in the different honeys are a consequence of their botanical source, also influenced by the harvesting season (da Silva et al., 2016).

All studied honeys followed Portuguese and European legislations (Decreto Lei no 214/2003; Council directive 2001/110/EC, 2002), which allow a maximum of 50 meq/kg for the free acid content, indicating the absence of undesirable fermentations. Statistical analysis regarding both free and total acidity among the analyzed samples is detailed in Fig. 3 and Fig. S2.

Carob tree, bell heather and raspberry honeys had the highest values of free acidity (20.8–27.7 meq/kg), whilst incense honeys presented the lowest values (7.5–11.6 meq/kg), being in accordance with the regulation for Azores honey (Commission Delegated Regulation (EU) PDO-PT-0268-AM01, 2019), which assigns low acidity values for this honey type. The values obtained for carob tree honeys were slightly higher than those reported by Terrab et al. (2003) for Moroccan honeys, although lower than that of other Portuguese honeys (Aazza et al., 2013). Free acidity values previously reported for Portuguese bell heather honey (Karabagias et al., 2018), were slightly superior than those obtained in this work.

The free acidity value of chestnut honey sample C2 (11.7 meq/kg), was inferior to that reported also for Portuguese chestnut honey (Karabagias et al., 2018).

Eucalyptus honeys showed similar free acidity values (14.7–17.2 meq/kg) to those found for other Portuguese eucalyptus honeys (Karabagias et al., 2018).

Orange honeys free acidity values (12.2–15.7 meq/kg) were similar to those reported for Greek orange honeys, although higher when compared to Egyptian, Moroccan and Spanish honeys (Karabagias et al., 2017).

Free acidity values for sunflower honeys ranged between 17.6 and 21.4 meq/kg, being similar to those reported by Sakač et al. (2019) on Serbian and Italian honeys, although Mendes et al. (1998) reported values slightly lower for Portuguese honey.

3.3.7. Diastase activity (DA)

DA is a parameter representing freshness and/or overheating of honey since it decreases with time and temperature. Its levels may reflect differences in the botanical source or geographic origin (Aazza et al., 2013).

DA values ranged from 0.1 to 23.2 shade units/g, with pairwise comparisons between honey samples from different botanical source showing significant differences ($p < 0.05$), as detailed in Fig. S2. According to the legislations (Decreto Lei no 214/2003; Council directive 2001/110/EC, 2002) several honeys showed unacceptable DA (<8 Schade units/g honey). However, the same regulations state that honeys with naturally low enzyme activity, like citrus honey, stand a minimum of 3 Schade units if the HMF content is lower than 15 mg/kg. However, with little processing, blending or storage, HMF can easily reach or exceed the limit of 15 mg/kg, being suggested that such rules should be reviewed, taking into account the honeys that are naturally low in enzymes (Thrasyvoulou et al., 2018). In this study the samples with lower DA included incense, lavender, orange, and strawberry tree honeys. These last honeys specifically, St1 and St2, showed values closer to 0, similar to the results described by (Oddo and Piro, 2004) for *Arbutus* honey with a range between 0 and 9.7 Schade units/g. The high value obtained for St4 (10.7) could be explained by the lower HMF when comparing to the other strawberry tree honeys, as well to the different geographic production location. Honeys St1 and St2 were obtained in Baixo Alentejo, characterized by a hot and dry climate, and St4 in Algarve, specifically in Aljezur region, with wetter conditions, near the Atlantic Ocean (Fig. 1).

3.3.8. Proline content

Proline represents 50–80% of the total amino acids content in honey, being considered as a maturation criterion as well for assessing sugar adulteration (Pita-Calvo and Vázquez, 2017). According to Bogdanov et al. (1999) a minimum of 0.180 mg/g of proline is accepted as the limit for genuine honey, which was verified in the analyzed honeys (Table 2).

The proline content in the studied honeys ranged from 0.2 to 2.1 mg/g. Pairwise comparisons between honey types, showed that some of them exhibited significant differences ($p < 0.05$) according to botanical source. Carob tree honeys presented significant differences when comparing to the other honey types, showing a higher proline content, except with bell heather and strawberry tree honeys (Fig. S2). Darker honeys such as those obtained from carob tree or bell heather showed higher proline content while lighter honeys, specifically incense, lavender or orange exhibited lower values (Table 2).

3.3.9. Sugar profile

Fructose and glucose account for>60% of the sugars present in nectar honey, while honeydew honeys contain lower values of these monosaccharides, but higher levels of oligosaccharides, such as melezitose and erlose (Tomás et al., 2017).

In this study, the sum of fructose and glucose content, 61%-78% (Table 3), was in agreement with Portuguese and European requirements for nectar honeys (Decreto Lei no 214/2003; Council directive 2001/110/EC, 2002). Pairwise comparisons between honey types showed no significant differences ($p < 0.05$) in fructose content amongst honey samples. Nevertheless, eucalyptus honeys exhibited significant differences towards incense, orange, and sunflower honeys concerning glucose content (Fig. S3).

Minor sugars showed significant differences between botanical sources, mainly isomaltose and maltulose (Fig. 4). Maltulose (0.5–6.8%) and turanose (0.4–3.1%) were found in all the analyzed honeys. Isomaltose (0.2–6.7%) and maltose (0.4–3.2%) were detected in most of the honeys and trehalose ranged from 0.3 to 2.5%, despite not being detected in chestnut honey. Maltose and maltulose content in eucalyptus honey showed significant differences compared to incense, orange, and

sunflower honeys (Figs. 4 and S3). Eucalyptus honey also evidenced higher isomaltose content, with significant differences compared to the other honey types (Fig. 4). Orange honeys presented a higher amount of trehalose, significantly different from that obtained for sunflower and incense honeys. Orange honeys also had a higher turanose content, which was statistically different from that of sunflower honeys (Fig. 4).

Sucrose was only detected in a few of the honeys, and the values obtained were within the defined limits (<5%), indicating the absence of adulterations with sugar syrups (Soares et al., 2017). In this case, pairwise comparison was only performed for honey types containing sucrose (Fig. S3), showing no significant differences between the analyzed honeys ($p < 0.05$).

Other less abundant sugars also detected were the disaccharide melibiose, and the trisaccharides melezitose, raffinose and erlose. Melibiose was only detected in one sample of orange honey (O2), melezitose was found in one sample of carob tree honey (Ct3) and in another of lavender honey (L5) whilst raffinose was absent in all honeys. These last two trisaccharides are characteristic in honeydew honeys (Rodríguez-Flores et al., 2019a). Erlose was mainly detected in lavender honeys, with a maximum value of 1.8%. Similar results were obtained by Tomás et al. (2017) for Portuguese lavender honeys.

3.3.10. Relevant physicochemical parameters for the differentiation between monofloral honeys

Pairwise comparisons between honey types (Fig. 3 and Fig. S2), evidenced some physicochemical parameters that could be considered potential good discriminators of monofloral honeys according to botanical source. Electrical conductivity, colour index, free and total acidity as well diastase activity showed significant differences among some honey types. The obtained results evidenced that some honey pairs could be differentiated by all these parameters, namely carob tree honey from those of lavender, orange and incense, or bell heather honey from those of lavender, orange and incense, or eucalyptus honey from that of incense and lavender honey from that of sunflower.

4. Conclusions

Portugal produces a great variety of monofloral honey types all over the mainland, and in Azores islands, with different composition and physicochemical profile, both influenced by the richness of the surrounding flora. Currently there is a new generation of consumers driven by environment sustainability as well as demanding high-quality. To achieve these market requirements, as well to combat honey adulteration, it is very important to invest on several studies to validate the quality and authenticity of Portuguese monofloral honeys. Knowledge on these honeys is scarce and this work aimed to fill gaps in information and produce a sustained recommendation on methods, parameters and needs to be met in the future. While in complementary studies the significance of other quality parameters, such as phenolic or volatile composition, is being evaluated, the present study showed these honeys to be microbiologically safe and, in addition to the relevance of the sugar profile, allowed to define a set of physicochemical parameters, namely electrical conductivity, colour, free and total acidity, as well diastase activity as important tools that are more likely to provide additional information in the differentiation of monofloral honeys.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.111362.

References

Aazza, S., Lyoussi, B., Antunes, D., & Miguel, M. G. (2013). Physicochemical characterization and antioxidant activity of commercial Portuguese honeys. *Journal of Food Science, 78*(8), C1159–C1165. https://doi.org/10.1111/1750-3841.12201

Alves, A., Ramos, A., Gonçalves, M. M., Bernardo, M., & Mendes, B. (2013). Antioxidant activity, quality parameters and mineral content of Portuguese monofloral honeys. *Journal of Food Composition and Analysis, 30*(2), 130–138. https://doi.org/10.1016/j.jfca.2013.02.009

Anjos, O., Santos, A. J. A., Paixão, V., & Estevinho, L. M. (2018). Physicochemical characterization of *Lavandula* spp. honey with FT-Raman spectroscopy. *Talanta, 178*, 43–48. https://doi.org/10.1016/j.talanta.2017.08.099

Arroyo, T. M., González-Porto, A. V., & Esteban, C. B. (2017). Viper's bugloss (*Echium* spp.) honey typing and establishing the pollen threshold for monofloral honey. *PLoS ONE, 12*(10), Article e0185405. https://doi.org/10.1371/journal.pone.0185405

Bobis, O., Moise, A. R., Ballesteros, I., Reyes, E. S., Durán, S. S., Sánchez-Sánchez, J., & Alvarez-Suarez, J. M. (2020). Eucalyptus honey: Quality parameters, chemical composition and health-promoting properties. *Food Chemistry, 325*, Article 126870. https://doi.org/10.1016/j.foodchem.2020.126870

Bogdanov, S., Lüllmann, C., Martin, P., Ohe, W. Von der, Russmann, H., Vorwohl, G., Persano Oddo, L., Sabatini, A.-G., Marcazzan, G. L., Piro, R., Flamini, C., Morlot, M., Lhéritier, J., Borneck, R., Marioleas, P., Tsigouri, A., Kerkvliet, J., Ortiz, A., … & Vit., P. (1999). Honey quality and international regulatory standards: Review by the International Honey Commission. *Bee World*, *80*(2), 61–69. 10.1080/0005772X.1999.11099428.

Bogdanov, S., Martin, P., Lüllmann, C., Borneck, R., Flamini, Ch. C., Morlot, M., & Ivanov, T. (2002). *Harmonized methods of the International Honey commission (IHC) responsible for the methods*, 1–62.

Commission Delegated Regulation (EU) PDO-PT-0268-AM01 relating to "Mel dos Açores" (2019) Official Journal of the European Union C 384/16.

Council directive 2001/110/EC relating to honey (2002) Official Journal of the European Communities L10/47.

da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry, 196*, 309–323. https://doi.org/10.1016/j.foodchem.2015.09.051

Decreto Lei no 214/2003 de 18 de Setembro do Ministério da Agricultura, Desenvolvimento Rural e Pescas. Diário da República: I série-A, No 216 (2003).

DOOR (2021) The Database of Origin & Registration (DOOR) from European Commission. (2021). *eAmbrosia - the EU geographical indications register*. Retrieved from http://ec.europa.eu/agriculture/quality/door/list.html. Accessed January 26, 2021.

El Menyiy, N., Dobre, I., Akdad, M., Elamine, Y., & Lyoussi, B. (2020). Microbiological quality, physicochemical properties, and antioxidant capacity of honey samples commercialized in the Moroccan Errachidia region. *Journal of Food Quality, 2020*, 1–9. https://doi.org/10.1155/2020/7383018

Escuredo, O., Dobre, I., Fernández-González, M., & Seijo, M. C. (2014). Contribution of botanical origin and sugar composition of honeys on the crystallization phenomenon. *Food Chemistry, 149*, 84–90. https://doi.org/10.1016/j.foodchem.2013.10.097

Escuredo, O., Silva, L. R., Valentão, P., Seijo, M. C., & Andrade, P. B. (2012). Assessing *Rubus* honey value: Pollen and phenolic compounds content and antibacterial capacity. *Food Chemistry, 130*(3), 671–678. https://doi.org/10.1016/j.foodchem.2011.07.107

Estevinho, L. M., Feás, X., Seijas, J. A., & Pilar Vázquez-Tato, M. (2012). Organic honey from Trás-Os-Montes region (Portugal): Chemical, palynological, microbiological and bioactive compounds characterization. *Food and Chemical Toxicology, 50*(2), 258–264. https://doi.org/10.1016/j.fct.2011.10.034

Farmacopeia Portuguesa 9.0 (2008) INFARMED - Autoridade Nacional do Medicamento e Produtos de Saúde: Lisboa, Portugal.

Feás, X., Pires, J., Estevinho, M. L., Iglesias, A., & Araujo, J. P. P. D. (2010). Palynological and physicochemical data characterisation of honeys produced in the Entre-Douro e Minho region of Portugal. *International Journal of Food Science & Technology, 45*(6), 1255–1262. https://doi.org/10.1111/j.1365-2621.2010.02268.x

Ferrauto, G., & Pavone, P. (2013). Palynological, physico-chemical and organoleptic characteristics of carob tree (*Ceratonia siliqua* L.) honey from Sicily. *International Journal of Food Science & Technology, 48*(8), 1596–1602. https://doi.org/10.1111/ijfs.12129

Finola, M. S., Lasagno, M. C., & Marioli, J. M. (2007). Microbiological and chemical characterization of honeys from central Argentina. *Food Chemistry, 100*(4), 1649–1653. https://doi.org/10.1016/j.foodchem.2005.12.046

Generalized Estimation Equation Solver. (2012). Package 'gee'. Retrieved from http://CRAN.R-project.org/package=gee. Accessed January 30, 2021 R package version 4.13-18.

Gomes, T., Feás, X., Iglesias, A., & Estevinho, L. M. (2011). Study of organic honey from the Northeast of Portugal. Molecules, 16(7), 5374–5386. 10.3390/molecules 16075374.

Homrani, M., Escuredo, O., Rodríguez-Flores, M. S., Fatiha, D., Mohammed, B., Homrani, A., & Seijo, M. C. (2020). Botanical origin, pollen profile, and physicochemical properties of Algerian honey from different bioclimatic areas. *Foods, 9*(7), 938. https://doi.org/10.3390/foods9070938

Hossain, L., Lim, L. Y., Hammer, K., Hettiarachchi, D., & Locher, C. (2021). Honey-based medicinal formulations: A critical review. *Applied Sciences, 11*(11), 5159. https://doi.org/10.3390/app11115159

ISO 21528-2:2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of Enterobacteriaceae - Part 2: Colony-count technique.

ISO 21527-2:2008. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of yeasts and moulds - Part 2: Colony count technique in products with water activity less than or equal to 0,95.

ISO 15213:2003. Microbiology of food and animal feeding stuffs -Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions.

Juan-Borrás, M., Domenech, E., Hellebrandova, M., & Escriche, I. (2014). Effect of country origin on physicochemical, sugar and volatile composition of acacia, sunflower and tilia honeys. *Food Research International, 60*, 86–94. https://doi.org/10.1016/j.foodres.2013.11.045

Juan-Borrás, M., Periche, A., Domenech, E., & Escriche, I. (2015). Correlation between methyl anthranilate level and percentage of pollen in Spanish citrus honey. *International Journal of Food Science & Technology, 50*(7), 1690–1696. https://doi.org/10.1111/ijfs.12827

Karabagias, I. K., Louppis, A. P., Karabournioti, S., Kontakos, S., Papastephanou, C., & Kontominas, M. G. (2017). Characterization and geographical discrimination of commercial *Citrus* spp. Honeys produced in different Mediterranean countries based on minerals, volatile compounds and physicochemical parameters, using chemometrics. *Food Chemistry, 217*, 445–455. https://doi.org/10.1016/j.foodchem.2016.08.124

Karabagias, I. K., Maia, M., Karabagias, V. K., Gatzias, I., & Badeka, A. V. (2018). Characterization of eucalyptus, chestnut and heather honeys from Portugal using multi-parameter analysis and chemo-calculus. *Foods, 7*(12), 194. https://doi.org/10.3390/foods7120194

Karapetsas, A., Voulgaridou, G.-P., Iliadi, I., Tsochantaridis, I., Michail, P., Kynigopoulos, S., Lambropoulou, M., Stavropoulou, M.-I., Stathopoulou, K., Karabournioti, S., Aligiannis, N., Gardikis, K., Galanis, A., Panayiotidis, M. I., & Pappa, A. (2020). Honey extracts exhibit cytoprotective properties against UVB-induced photodamage in human experimental skin models. *Antioxidants, 9*(7), 566. https://doi.org/10.3390/antiox9070566

Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. *Bee World, 59*(4), 139–157. https://doi.org/10.1080/0005772X.1978.11097714

Makhloufi, C., Abderrahim, L. A., & Taibi, K. (2021). Characterization of some Algerian honeys belonging to different botanical origins based on their physicochemical properties. *Iranian Journal of Science and Technology, Transaction A: Science, 45*, 189–199. https://doi.org/10.1007/s40995-020-01047-3

Mendes, E., Brojo Proença, E., Ferreira, I. M. P. L. V. O., & Ferreira, M. A. (1998). Quality evaluation of Portuguese honey. *Carbohydrate Polymers, 37*(3), 219–223. https://doi.org/10.1016/S0144-8617(98)00063-0

NP 4405:2002. Microbiologia Alimentar. Regras gerais para a contagem de microrganismos. Contagem de colónias a 30°C.

Oddo, L., & Piro, R. (2004). Main European unifloral honeys: Descriptive sheets. *Apidologie, 35*(Suppl. 1), S38–S81. https://doi.org/10.1051/apido:2004049

Pauliuc, D., Dranca, F., & Oroian, M. (2020). Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters suitability for Romanian honey authentication. *Foods, 9*(3), 306. https://doi.org/10.3390/foods9030306

Perez-de-Zabalza A. (1992) Análisis polínico de mieles de los valles pirenaicos navarros (España). In *Actes del Simposi Internacional de Botànica "Pius Font i Quer" Vol. II Fanerogàmia, 1988, September* (pp. 183-187). Lleida: Institut d́Estudis Ilerdencs.

Pita-Calvo, C., & Vázquez, M. (2017). Differences between honeydew and blossom honeys: A review. *Trends in Food Science & Technology, 59*, 79–87. https://doi.org/10.1016/j.tifs.2016.11.015

Rodopoulou, M. A., Tananaki, C., Kanelis, D., Liolios, V., Dimou, M., & Thrasyvoulou, A. (2021). A chemometric approach for the differentiation of 15 monofloral honeys based on physicochemical parameters. *Journal of the Science of Food and Agriculture, 17*(10), 1–8. https://doi.org/10.1002/jsfa.11340

Rodríguez-Flores, M. S., Escuredo Pérez, O., & Seijo Coello, M. C. (2014). Characterization of *Eucalyptus globulus* honeys produced in the Eurosiberian area of the Iberian Peninsula. *International Journal of Food Properties, 17*(10), 2177–2191. https://doi.org/10.1080/10942912.2013.790050

Rodríguez-Flores, M. S., Escuredo, O., Míguez, M., & Seijo, M. C. (2019a). Differentiation of oak honeydew and chestnut honeys from the same geographical origin using chemometric methods. *Food Chemistry, 297*, Article 124979. https://doi.org/10.1016/j.foodchem.2019.124979

Rodríguez-Flores, M. S., Escuredo, O., Seijo-Rodríguez, A., & Seijo, M. C. (2019b). Characterization of the honey produced in heather communities (NW Spain). *Journal of Apicultural Research, 58*(1), 84–91. https://doi.org/10.1080/00218839.2018.1495417

Sakač, M. B., Jovanov, P. T., Marić, A. Z., Pezo, L. L., Kevrešan, Ž. S., Novaković, A. R., & Nedeljković, N. M. (2019). Physicochemical properties and mineral content of honey samples from Vojvodina (Republic of Serbia). *Food Chemistry, 276*, 15–21. https://doi.org/10.1016/j.foodchem.2018.09.149

Sinacori, M., Francesca, N., Alfonzo, A., Cruciata, M., Sannino, C., Settanni, L., & Moschetti, G. (2014). Cultivable microorganisms associated with honeys of different geographical and botanical origin. *Food Microbiology, 38*, 284–294. https://doi.org/10.1016/j.fm.2013.07.013

Soares, S., Amaral, J. S., Oliveira, M. B. P. P., & Mafra, I. (2017). A comprehensive review on the main honey authentication issues: Production and origin. *Comprehensive Reviews in Food Science and Food Safety, 16*(5), 1072–1100. https://doi.org/10.1111/1541-4337.12278

Terrab, A., Díez, M. J., & Heredia, F. J. (2003). Palynological, physico-chemical and colour characterization of Moroccan honeys: III. Other unifloral honey types. *International Journal of Food Science & Technology, 38*(4), 395–402. https://doi.org/10.1046/j.1365-2621.2003.00713.x

Thrasyvoulou, A., Tananaki, C., Goras, G., Karazafiris, E., Dimou, M., Liolios, V., Kanelis, D., & Gounari, S. (2018). Legislation of honey criteria and standards. *Journal of Apicultural Research, 57*(1), 88–96. https://doi.org/10.1080/00218839.2017.1411181

Tomás, A., Russo-Almeida, P., & Vilas-Boas, M. (2017). Avaliação do perfil de açúcares do mel de rosmaninho Português. *Revista de Ciências Agrárias, 40*(SPE), 261–270. https://doi.org/10.19084/RCA16211

Von der Ohe, W., Persano Oddo, L., Piana, M. L., Morlot, M., & Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie, 35*(Suppl. 1), S18–S25. https://doi.org/10.1051/apido:2004050