



UNIVERSITÀ POLITECNICA DELLE MARCHE
Repository ISTITUZIONALE

Chemometric approach to the analysis of antioxidant properties and colour of typical Italian monofloral honeys.

This is the peer reviewed version of the following article:

Original

Chemometric approach to the analysis of antioxidant properties and colour of typical Italian monofloral honeys / Castiglioni, Sara; Stefano, Mariassunta; Astolfi, Paola; Carloni, Patricia. - In: INTERNATIONAL JOURNAL OF FOOD SCIENCE & TECHNOLOGY. - ISSN 1365-2621. - STAMPA. - 52:5(2017), pp. 1138-1146. [10.1111/ijfs.13397]

Availability:

This version is available at: 11566/245132 since: 2022-05-25T15:40:00Z

Publisher:

Published

DOI:10.1111/ijfs.13397

Terms of use:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.

This item was downloaded from IRIS Università Politecnica delle Marche (<https://iris.univpm.it>). When citing, please refer to the published version.

(Article begins on next page)

Chemometric approach to the analysis of antioxidant properties and colour of typical Italian monofloral honeys.

RUNNING TITLE. Monofloral honeys antioxidant activity & colour

Sara Castiglioni^a, Mariassunta Stefano^b, Paola Astolfi^c, Patricia Carloni^{a,*}

^a Dipartimento di Scienze Agrarie, Alimentari e Ambientali - D3A, Università Politecnica delle Marche, 60131 Ancona, Italy

^b ASSAM, Agenzia per i Servizi nel Settore Agroalimentare delle Marche, Centro Agrochimico Regionale - Jesi (AN), 60035, Italy

^c Dipartimento Scienze e Ingegneria della Materia, dell'Ambiente ed Urbanistica - SIMAU, Università Politecnica delle Marche, 60131 Ancona, Italy

Corresponding author:

Prof. Patricia Carloni

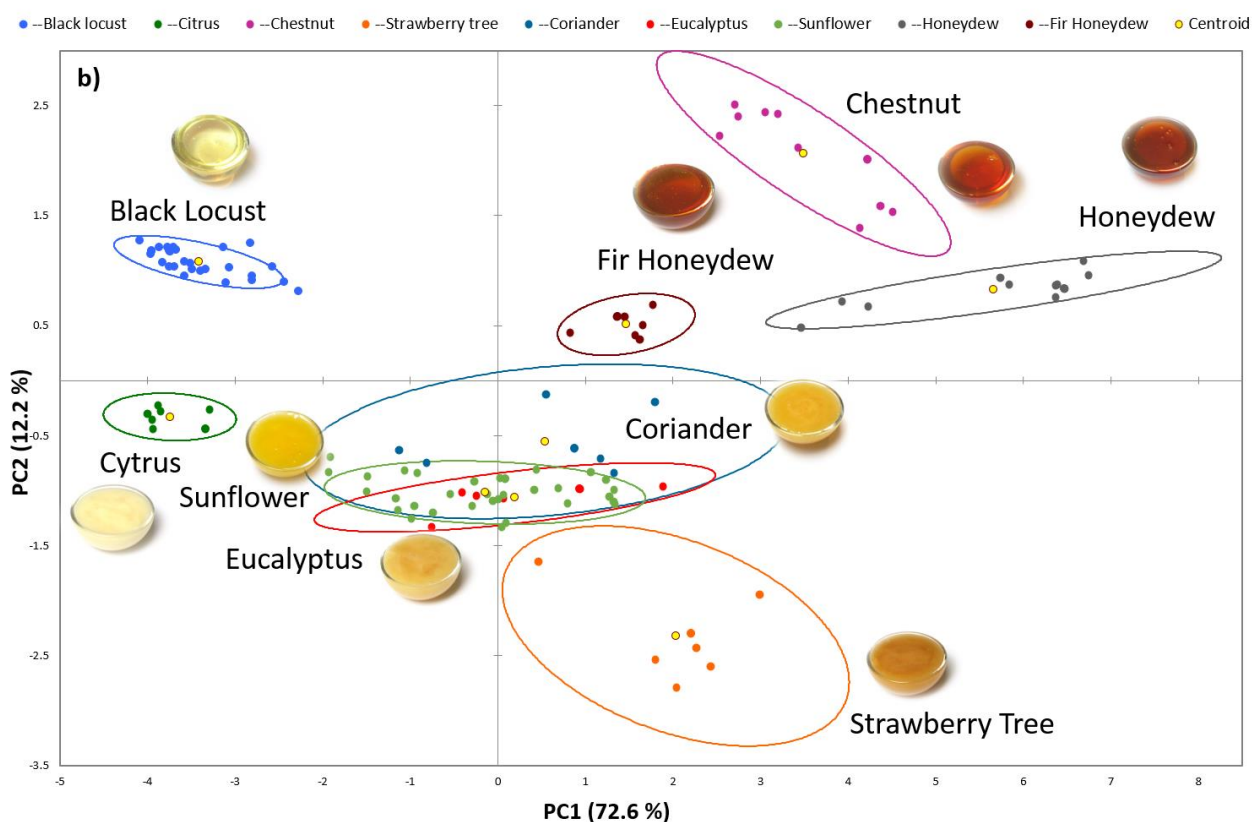
Tel. +39 071 2204739 - Fax +39 071 2204988

E-mail: p.carloni@staff.univpm.it

Abstract

The antioxidant and colour properties of nine types of monofloral Italian honeys were analysed and correlated with multivariate analysis to find relationships able to characterize the honey floral source. Total phenolic content (TPC) and antioxidant activity of the honey samples increase in the order Citrus \approx Black Locust < Sunflower \approx Eucalyptus \approx Coriander < Fir Honeydew \approx Chestnut < Honeydew \approx Strawberry Tree and mainly correlate with colour. A Linear Discriminant Analysis was carried out to choose spectrophotometric λ able to characterize the colour of the different types of honey. Elaboration of data with Cluster and Principal Component Analysis enables differentiation among the floral source of some honey samples, namely Strawberry Tree, Honeydew, Citrus, Black Locust, Chestnut and Fir Honeydew.

Graphical Abstract



Keywords

Honey; Colour; Polyphenols; Folin-Ciocalteu Method; Antioxidant Activity; Chemometrics; PCA.

1. Introduction

Honey is a natural food produced from nectar or secretions of plants or from excretions of plant-sucking insects by *Apis mellifera* L. bees. This food contains more than 200 compounds and is considered as one of the most complete nourishments for humans (Bueno-Costa et al. 2016): its main components are fructose and glucose, but other constituents such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, aromatic substances, flavonoids and other phytochemicals are also present (da Silva et al. 2016).

In the last few years the use of natural dietary antioxidants as effective protection against oxidative damage has become very popular. In fact, several epidemiological studies have indicated that a diet rich in phytochemical antioxidants is able to prevent or retard chronic diseases (Slavin and Lloyd 2012). Consequently, the interest in the identification and quantification of these compounds in honey samples has significantly increased, and has led to demonstrate that phenolic compounds (especially flavonoids) constitute the most important class of compounds with antimicrobial, anti-inflammatory, antimutagenic, antitumor, and antioxidative properties besides other beneficial effects on human health (Alvarez-Suarez et al. 2010). Several authors have demonstrated that the antioxidant activity of honey varies significantly according to its floral source (Chaikham et al. 2016) probably because nectar and excretions of plants contain different polyphenolic compounds and bees transfer these bioactive compounds from plants to honey.

Hundreds of different unifloral honeys are known, and at least half of them can be produced in Italy (Osservatorio Nazionale Miele 2013) because of the appropriate geographical and climatic conditions for apiculture. Unifloral honeys have high demand and commercial value on the market for their particular sensory characteristics and Italian consumers show strong positive preferences for locally produced unifloral honeys (Cosmina et al. 2016).

There are several reports concerning monofloral honeys produced from different countries, but

despite the great diversity of honeys produced in Italy, limited studies (Perna et al. 2013, Rosa et al. 2011) have been carried out on the characterisation of their antioxidant activity and on the correlation of this property with their botanical origin and colour.

Because some studies (Di Bella et al. 2015, Truzzi et al. 2014, Zhao et al. 2016) suggest the use of multivariate analysis for a more reliable characterization of the botanical origin of honey, the aim of this study was therefore to determine the antioxidant and colour properties of Italian honeys from different botanical origins and to correlate them with multivariate analysis to find relationships able to characterize them.

2. Material and methods

2.1 Chemicals and equipment

All chemicals were of the highest analytical grade. [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), gallic acid (GA), Folin-Ciocalteu reagent (2N solution), potassium persulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH•) were purchased from Sigma-Aldrich Chemical Co. (Milan, Italy). Ultrapure water was generated from a Milli-Q system by Merck Millipore (Merck KGaA, Darmstadt, Germany) and was used for all the experiments. Spectrophotometric measurements were recorded in quadruplicate on a microplate reader (Synergy HT, Biotek, Winooski, VT, USA). Electron Paramagnetic Resonance (EPR) spectra were recorded in duplicate on a Bruker EMX spectrometer (Bruker, Karlsruhe, Germany) operating at X-Band equipped with an XL microwave frequency counter and a NMR Gaussmeter for the calibration of the magnetic field. Data represent average values from at least three independent experiments (n=3). Colour measurements were performed with an optical Lovibond® Comparator System 2000 (The Tintometer Ltd, Amesbury, UK).

2.2 Honey samples

A total of 117 monofloral honey samples [28 Black Locust (*Robinia pseudoacacia* L.), 7 Citrus (*Citrus* spp.), 33 Sunflower (*Helianthus annuus* L.), 7 Coriander (*Coriandrum sativum* L.), 7 Eucalyptus (*Eucalyptus* spp.), 7 Strawberry Tree (*Arbutus unedo* L.), 10 Chestnut (*Castanea sativa* Mill.), 7 Fir Honeydew (Fir: *Abies alba* Mill. and/or *Picea abies* L.), and 11 Honeydew (Forest Honeydew, *Insect: Metcalfa pruinosa* (Say))] were directly collected by beekeepers during the 2012, 2013 and 2014 harvest seasons in different Italian regions (Figure 1). Honeys were of high quality and were selected for the annual contests “Premio Qualità Miele Marchigiano” (samples from Marche) or “Grandi Mieli d’Italia Tre Gocce d’Oro”: these were provided by ASSAM (Agenzia per i Servizi nel Settore Agroalimentare delle Marche) and by a private consulting company (Piana Ricerca e Consulenza srl) specialized in the field of beekeeping. The providers confirmed the honey botanical origin by mellissopalynological analysis (Persano Oddo et al. 1995) or by sensory analysis and analysed their moisture and HMF content to verify the respect of the limits according to the standards set by the 2001/110/EC regulation (European Economic Community 2001). Samples were stored in polyethylene tubes at 4°C in the dark until used and were received around six months after the harvesting. All the analysis were carried out by six to twelve months from the harvesting and crystallization state was checked visually at reception to classify (see Supplementary Material, Table S1) any sample as liquid (1) or crystallized (0).

2.3 Colour analysis (Lovibond)

The colour of the honey samples was measured with an optical comparator. Approximately 3 g of each honey sample were poured into the sample holder and were heated (max 65°C) to dissolve sugar crystals. The sample colour was visually matched in the comparator instrument against Pfund graded coloured glass filters.

2.4 Spectrophotometric measurement for colour analysis

To measure the colour of the honey samples, spectrophotometric measurements in the visible

range were also carried out. Briefly, 200 μ L of a 50% w/v honey solution were added in each well of a transparent 96-well microplate and the absorbance visible spectrum (350–700 nm) was recorded at constant intervals ($\Delta\lambda = 5$). The results were expressed as AU (Arbitrary Units).

The obtained data were statistically elaborated by discriminant analysis (DA) to identify the set of "best discriminating" λ variables between groups of honeys of different botanical origin (see Results).

2.5 *Total phenolic content (TPC)*

Total phenolic content in the honey samples was determined using the Folin-Ciocalteu reagent (Singleton et al. 1999). Briefly, in each well of a transparent 96-well microplate, 50 μ L of a 5% w/v honey solution, or of a 60 mM Gallic Acid standard ethanolic solution appropriately diluted (0 - 0.50 mM in water), were added followed by 150 μ L of a 10-fold diluted solution of the Folin–Ciocalteu reagent. The microplate was shaken and left to stand for 10 min in the dark. After this time, 100 μ L of a 10% Na₂CO₃ water solution were added to each well. Samples were left to stand for 120 min at room temperature in the dark and then absorbance was read at 760 nm against water as blank. The results are expressed as mg Gallic Acid Equivalents (mg GAE)/kg honey.

2.6 *Antioxidant activity (AA)*

2.6.1 *ABTS assay*

The antioxidant activity of the different honey samples was determined using the ABTS assay (Re et al. 1999). The coloured radical cation (ABTS^{•+}) was prepared by mixing a 7.0 mM aqueous ABTS solution with a 24.5 mM aqueous solution of K₂S₂O₈ as oxidizing agent in a 9:1 ratio respectively, and allowing the mixture to stand at room temperature in the dark for 12-16 h before use. The prepared ABTS^{•+} stock solution was then diluted \approx 50-fold with water to reach an absorbance of 0.9 ± 0.1 at 734 nm. For the assay, 30 μ L of a 2.5 or 5% w/v honey solution (depending on the kind of honey) or of a 1.8 mM Trolox standard ethanolic solution appropriately diluted (0 - 0.30 mM in

water), or water as control, were added in each well of a transparent 96-well microplate, followed by 270 μ L of the diluted ABTS^{•+} solution. The microplate was shaken and left to stand for 120 min at room temperature in the dark; after this time the absorbance of the solution was read at 734 nm against water as blank. The antioxidant activity was determined as inhibition percentage using the following equation:

$$\% \text{ Inhibition } A_{734} = (1 - A_s/A_c) \times 100$$

where: A_s is the absorbance at 734 nm of samples containing honey or standard; A_c is the absorbance of the control. The results are expressed as mmol Trolox Equivalents (mmol TXE)/kg honey.

2.6.2 DPPH assay

The antioxidant activity of honey samples was also assessed using the DPPH assay (Prior et al. 2005) monitored by reading the absorbance of the radical at 517 nm (DPPH-Vis Method) or by recording its EPR signal (DPPH-EPR Method).

For the assay, 100 μ L of a 2, 2.5 or 5% w/v honey solution (depending on the kind of honey), or of a 0.45 mM Trolox standard ethanolic solution appropriately diluted (0 - 0.15 mM in water), or water as control, were mixed with 200 μ L of a 0.2 mM ethanolic DPPH[•] solution. After 15 min at room temperature in the dark, the absorbance of the solution at 517 nm was read against water as blank on a transparent 96-well microplate (DPPH-Vis), or the area of the DPPH[•] EPR spectrum was recorded (DPPH-EPR). For EPR measurements, samples were transferred to 50 μ L capillary tubes and the following instrumental settings were used: frequency 9.78 GHz, power 25 mW, modulation amplitude 2 Gauss, gain 5×10^5 , field width 100 G, time constant 0.64 ms, scan time 21 s.

The DPPH[•] scavenging activity was determined as inhibition percentage using the following equation:

$$\% \text{ Inhibition} = (1 - A_s/A_c) \times 100$$

where A_s is the absorbance at 517 nm or the area of the EPR signal of the solution containing honey samples or standard and A_c of the control lacking honey. The results are expressed as mmol Trolox Equivalents (mmol TXE)/kg honey.

2.7 Data analysis

Appropriate controls were carried out in all the experiments described above. The results of TPC, ABTS, DPPH-Vis, DPPH-EPR tests were expressed as mean values from at least three independent experiments ($n=3$). Honey samples were classified according to their botanical origin and the results were expressed as mean with standard deviation (SD) for the different classes. Statistical differences were obtained through an analysis of variance (ANOVA) followed by Tukey's multiple comparison test at 95% confidence level ($p \leq 0.05$). Normality was checked with the Jarque-Bera procedure and homogeneity of the variance with the Box test; if the assumption of homogeneity of the variance was not acceptable, the Kruskal-Wallis test was used as an alternative to the ANOVA.

The results were also processed using multivariate chemometric techniques involving discriminant analysis (DA), cluster analysis (CA) and principal component analysis (PCA). All statistical treatments were performed using XLSTAT software (Addinsoft SARL).

3. Results

3.1 Colour analysis (Lovibond)

The colour of honey samples was measured with an optical comparator and the obtained values result in the range reported in the literature for each type of monofloral honey (Persano Oddo et al. 2004, Petretto et al. 2015): in particular, it can be only noticed the presence of a sample with a very high value (85 mmPfund) within Strawberry Tree (most 65-70 mmPfund) and another with a very low value (55 mmPfund) within Chestnut (most 70-80 mmPfund) honeys (see Supplementary Material, Table S1). The average values for each botanical origin are reported in Table 1 where the honey types are ordered from the lightest to the darkest.

3.2 *Spectrophotometric measurement for colour analysis*

Many authors report the use of absorption at 450 or at 635 nm to determine the colour of honey (Beretta et al. 2005, Ferreira et al. 2009). In this paper, a multivariate approach was used to better characterize this parameter. The visible absorption spectra from 350 to 700 nm of the honey samples were recorded, and data were elaborated using discriminant analysis (DA) to find variables that best discriminate between groups of honey from different botanical origin. A forward stepwise selection of λ variables was performed choosing the variables with the highest F-to-enter value and using a quadratic model, a tolerance threshold of 0.01 and a probability associated with each of the classes equal to the frequency. With this DA model (see Supplementary Material – Table S2), seven variables namely λ_{350} , λ_{360} , λ_{365} , λ_{385} , λ_{445} , λ_{490} , λ_{645} that correctly classify 100% of the honey samples were chosen.

3.3 *Total phenolic content (TPC)*

TPC mean values of honeys grouped by botanical origin are reported in Table. Significant higher phenolic contents are found in the bitter (Persano Oddo et al. 1995) Strawberry Tree and in the dark Honeydew honeys while the light honeys, Citrus and Black Locust, show significantly lower mean TPC. Honeys from Chestnut and Fir Honeydew show high TPC significantly different from Sunflower, Coriander and Eucalyptus honeys. The TPC values of honey samples mediated by botanical origin follow the same order of the Lovibond values ($r = 0.846$, $p < 0.0001$), except for Strawberry Tree honeys that have the highest polyphenolic content despite their colour which is lighter than both Honeydew and Fir Honeydew honeys. In addition, it can be observed the large variability in the data obtained for Honeydew honeys (583 - 1039 mg GAE/kg) and the presence of a sample with a very low TPC within Strawberry Tree (563.3 mg GAE/kg) honey samples (see Supplementary Material, Table S1).

3.4 *Antioxidant activity levels*

At present there is no universal method to test the antioxidant activity of a food matrix due to the large variability of compounds and to the several reaction mechanisms involved; for this reason, it is recommended to test the studied samples with different methods and to compare the results (Amorati and Valgimigli 2015, Gülçin 2012). In the present study, the antioxidant activity was determined by ABTS and DPPH assays: the decay of DPPH• radical was monitored using both by spectrophotometric and EPR techniques.

3.4.1 ABTS assay

The antioxidant activity (AA) of the honey samples was assessed using the reaction of the ABTS^{•+} with antioxidants. Because of the large difference in the antioxidant activity between samples, they were tested at different concentrations and the linearity of the response with the dilution was checked before the analysis.

The obtained results correlate quite well with total polyphenols content ($r = 0.7724$, $p < 0.0001$) and are reported in Table 1. The antioxidant activity of honeys varies with the honey's floral source, but the differences are less remarkable with respect to TPC. Noteworthy are the higher values obtained for Eucalyptus and Sunflower honeys with this assay, compared with those measured with the other tests.

3.4.2 DPPH assay

In the DPPH assay a stable nitrogen centred radical reacts with antioxidants by hydrogen/electron transfer at different rates and the disappearance of the DPPH• radical during the reaction can be monitored by a spectrophotometer (DPPH-Vis) or by a paramagnetic resonance spectrometer (DPPH-EPR) (Alvarez-Suarez et al. 2012). Also for this test, the antioxidant response with the concentration of the sample was linear.

As can be seen from the data reported in Table 1, the results obtained with both DPPH• monitoring methods are strictly correlated ($r = 0.9926$; $p < 0.0001$) and are of the same order of

magnitude. Moreover, they correlate both with antioxidant activity measured with the ABTS assay (DPPH-Vis: $r = 0.7114$, $p < 0.0001$; DPPH-EPR: $r = 0.7019$; $p < 0.0001$) and, to a major extent, with polyphenolic content (DPPH-Vis: $r = 0.8728$, $p < 0.0001$; DPPH-EPR: $r = 0.8603$; $p < 0.0001$).

4. Discussion

Analysis of the antioxidant activity and colour of the honey samples gives results in agreement with literature reports (Can et al. 2015, Wilczyńska 2014, Baek et al. 2015, Petretto et al. 2015, Persano Oddo et al. 1995) and shows that these proprieties vary with the botanical origin of honeys. In general, Strawberry Tree honeys show the highest values in all the tests, especially in the DPPH assay, despite their colour is not so dark (see Table 1); however, it can be noted that these honeys have a very bitter taste that can be attributed to the presence of large amount of polyphenols (Drewnowski and Gomez-Carneros 2000). On the opposite end, Citrus and Black Locust honeys, which are characterised by a light colour and a delicate flavour (Persano Oddo et al. 1995), have the lowest values in all the tests. Chestnut honeys possess a high antioxidant activity and a dark colour; Honeydew and Fir Honeydew, which unlike the other honeys are produced from a secretion of some scale insects and shaft resins, have high values in all the analyses including colour, although their antioxidant activity is lower than those of Strawberry Tree.

The obtained results indicate that the floral source of honeys influences their colour, antioxidant activity and sensory characteristics. However, small deviations in the correlation between the test likely due to the presence of various phytochemicals in different honeys prevents to get a simple relationship between these factors and suggests the possibility of classifying the honeys with multivariate analysis.

4.1 Multivariate analysis

Multivariate analysis was applied to all studied honey samples to establish a more simplified view of the relationship among the botanical source, antioxidant activity and colour parameters.

4.1.1 Cluster Analysis

First of all, a hierarchical cluster analysis was performed to verify if the data structure would be able to identify groups among the honey samples.

For the analysis, the whole data set incorporating all honey samples and all the variables analysed was used and the single linkage algorithm was applied using the Euclidean distance to space the cluster. Moreover, because the botanical origin of honey contributes to the sugar composition and consequently to the rapid, medium or slow granulation process (Belay et al. 2015), the crystallization state of the honey samples, determined after nearly six months from harvesting, was added as a further variable to help differentiating honey types.

The result obtained by cluster analysis (see Supplementary material – Table S3), presented as a dendogram (Figure 2), shows the presence of eleven honey clusters. Black Locust, Chestnut, Citrus, Fir Honeydew and Strawberry Tree honey samples are each grouped in its cluster, while Honeydew samples are separated into five close clusters; Eucalyptus, Coriander and Sunflower are instead grouped together into the same cluster.

The possibility to group most of the honey samples into separate clusters indicates that the data on the antioxidant, colour and crystallization properties of honeys contain useful information for classifying the samples.

4.1.2 Principal component analysis (PCA)

PCA is a convenient tool for reduction of data dimension and visualisation of similarities among samples, and provides a first evaluation of the classificatory efficiency of the variables considered. With this purpose, the results obtained in the different analyses were submitted to PCA together with spectrophotometric variables selected by DA analysis and crystallization state of the honey samples; moreover, the DPPH-Vis parameter was excluded due to its strict correlation with the DPPH-EPR variable.

This PCA model that uses the whole data set and twelve variables (Table 2) led to three significant, principal components (PC) with an eigenvalue > 1, that explained the 94% of the total system variability. Table 2 shows the variance explained and the loading matrix for the first three principal components extracted. The first factor PC1 (72.6%) includes most of the information deriving from the experimental results except crystallization state of the honeys that is instead mainly considered in PC2 (12.2%). In this factor also the contribute of ABTS variable becomes important while TPC is not considered. Finally, the third factor (PC3) accounts for polyphenol content and antioxidant activity in contrast with visible absorption at $\lambda > 400$ nm that contributes to the brown tonality of the honey (9.1%). The loading plot and the score plot of the first two factors are displayed in Figure 3 and show that the samples of different botanical origin are well differentiate, although some of the honey samples are overlapped and fit in different groups of honeys. Fir Honeydew, Honeydew and Chestnut honeys, characterized by a high polyphenolic content and a brown colour, are located in the upper right area of the graph. In contrast, Black Locust and Citrus honeys with their light colour and their low antioxidant activity are located in the left area of the graph and are separated only for their crystallization state. Sunflower, Eucalyptus and Coriander honeys are somewhat overlapped underlining their intermediate and similar characteristics despite the honeys with lower ABTS values (namely Coriander) are located higher in the chart of PC1 vs PC2 (Figure 3). Finally, Strawberry Tree honeys with their solid state and the higher ABTS values (mainly considered as negative in PC2) are located on the bottom right side of the graph (Figure 3). In addition, the score plot of PC2 vs PC3 (Supplementary material - Figure S1) evidences the low absorbance at $\lambda > 400$ nm and the high AA of this kind of honeys.

5. Conclusions

Nine types of honeys, obtained from different regions of Italy, were analysed providing an antioxidant and colour characterization of the main Italian honey types. In particular, Coriander

honey, typical of central Italy and rarely described in the literature, was described.

All the parameters evaluated were used for characterising and differentiating the various honeys. The use of multiple tests to determine the antioxidant activity of honeys allows to have a more reliable picture of the antioxidants present in the different samples since several phenolic compounds can react differently depending on the test used.

A DA analysis was carried out to choose spectrophotometric variables able to differentiate among the different types of honey.

Multivariate analysis of antioxidant activity, phenolic content, crystallization state and colour enables differentiation among the botanical origin of some of the honey samples, namely Black Locust, Citrus, Strawberry Tree, Chestnut, Honeydew and Fir Honeydew. In addition, the method confirms the validity of antioxidant and colour analysis together with the typical crystallization state as a tool for the characterisation and classification of honey samples with easy and low-cost instrumental techniques which require short times and can be used in routinely daily analysis.

Finally, monofloral Italian honeys, covering different regions and floral species, have been characterized in order to promote the production and the consumption of these bee products.

6. Acknowledgements

The authors thank the Polytechnic University of the Marche for financial support. Furthermore, the authors express their gratitude to Dr. Lucia Piana (Piana Ricerca e Consulenza srl) for supplying some of the honey samples.

7. References

Alvarez-Suarez, J. M., Giampieri, F., González-Paramás, A. M., Damiani, E., Astolfi, P., Martinez-Sanchez, G., Bompadre, S., Quiles, J. L., Santos-Buelga, C. & Battino, M. (2012). Phenolics from monofloral honeys protect human erythrocyte membranes against oxidative damage. *Food and Chemical Toxicology*, 50, 1508-1516.

- Alvarez-Suarez, J. M., Tulipani, S., Romandini, S., Bertoli, E. & Battino, M. (2010). Contribution of honey in nutrition and human health: a review. *Mediterranean Journal of Nutrition and Metabolism*, 3, 15-23.
- Amorati, R. & Valgimigli, L. (2015). Advantages and limitations of common testing methods for antioxidants. *Free Radical Research*, 49, 633-649.
- Baek, Y., Kim, Y. J., Baik, M.-Y., Kim, D.-O. & Lee, H. (2015). Total phenolic contents and antioxidant activities of Korean domestic honey from different floral sources. *Food Science and Biotechnology*, 24, 1453-1457.
- Belay, A., Solomon, W. K., Bultossa, G., Adgaba, N. & Melaku, S. (2015). Botanical origin, colour, granulation, and sensory properties of the Harennna forest honey, Bale, Ethiopia. *Food Chemistry*, 167, 213-219.
- Beretta, G., Granata, P., Ferrero, M., Orioli, M. & Maffei Facino, R. (2005). Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Analytica Chimica Acta*, 533, 185-191.
- Bueno-Costa, F. M., Zambiazzi, R. C., Bohmer, B. W., Chaves, F. C., Silva, W. P. d., Zanusso, J. T. & Dutra, I. (2016). Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. *LWT - Food Science and Technology*, 65, 333-340.
- Can, Z., Yildiz, O., Sahin, H., Akyuz Turumtay, E., Silici, S. & Kolayli, S. (2015). An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry*, 180, 133-141.
- Chaikham, P., Kemsawasd, V. & Apichartsrangkoon, A. (2016). Effects of conventional and ultrasound treatments on physicochemical properties and antioxidant capacity of floral honeys from Northern Thailand. *Food Bioscience*, 15, 19-26.

- Cosmina, M., Gallenti, G., Marangon, F. & Troiano, S. (2016). Attitudes towards honey among Italian consumers: A choice experiment approach. *Appetite*, 99, 52-58.
- 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.
- Di Bella, G., Lo Turco, V., Potortì, A. G., Bua, G. D., Fede, M. R. & Dugo, G. (2015). Geographical discrimination of Italian honey by multi-element analysis with a chemometric approach. *Journal of Food Composition and Analysis*, 44, 25-35.
- Drewnowski, A. & Gomez-Carneros, C. (2000). Bitter taste, phytonutrients, and the consumer: a review. *The American Journal of Clinical Nutrition*, 72, 1424-1435.
- European Economic Community. (2001). Council Directive relating to honey. 2001/110/EC Official Journal of the European Communities. L10. 47-52
- Ferreira, I. C. F. R., Aires, E., Barreira, J. C. M. & Estevinho, L. M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chemistry*, 114, 1438-1443.
- Gülçin, İ. (2012). Antioxidant activity of food constituents: an overview. *Archives of Toxicology*, 86, 345-391.
- Osservatorio Nazionale Miele. (2013). Italian honey a wealth of variety and richness. <http://www.informamiele.it/index.php/english/15-english/32-unifloral-honey/> Accessed 05/07/2016
- 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 & Technology*, 48, 1899-1908.
- Persano Oddo, L., Piazza, M. G., Sabatini, A. G. & Accorti, M. (1995). Characterization of unifloral honeys. *Apidologie*, 26, 453-465.

- Persano Oddo, L., Piro, R., with the collaboration, o., Bruneau, É., Guyot-Declerck, C., Ivanov, T., Piskulová, J., Flamini, C., Lheritier, J., Morlot, M., Russmann, H., Von der Ohe, W., Von der Ohe, K., Gotsiou, P., Karabournioti, S., Kefalas, P., Passaloglou-Katrali, M., Thrasyvoulou, A., Tsigouri, A., Marcazzan, G. L., Piana, M. L., Piazza, M. G., Sabatini, A. G., Kerkvliet, J., Godinho, J., Bentabol, A., Ortiz Valbuena, A., Bogdanov, S. & Ruoff, K. (2004). Main European unifloral honeys: descriptive sheets. *Apidologie*, 35, S38-S81.
- Petretto, G. L., Cossu, M. & Alamanni, M. C. (2015). Phenolic content, antioxidant and physico-chemical properties of Sardinian monofloral honeys. *International Journal of Food Science & Technology*, 50, 482-491.
- Prior, R. L., Wu, X. & Schaich, K. (2005). Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry*, 53, 4290-4302.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26, 1231-1237.
- 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, 1045-1053.
- Singleton, V. L., Orthofer, R. & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: *Methods in Enzymology* (edited by PACKER, L.). Pp. 152-178. New York, London: Academic Press.
- Slavin, J. L. & Lloyd, B. (2012). Health Benefits of Fruits and Vegetables. *Advances in Nutrition: An International Review Journal*, 3, 506-516.

- Truzzi, C., Illuminati, S., Annibaldi, A., Finale, C., Rossetti, M. & Scarponi, G. (2014). Physicochemical Properties of Honey from Marche, Central Italy: Classification of Unifloral and Multifloral Honeys by Multivariate Analysis. *Natural Product Communications*, 9, 1595-1602.
- Wilczyńska, A. (2014). Effect of filtration on colour, antioxidant activity and total phenolics of honey. *LWT - Food Science and Technology*, 57, 767-774.
- Zhao, J., Du, X., Cheng, N., Chen, L., Xue, X., Zhao, J., Wu, L. & Cao, W. (2016). Identification of monofloral honeys using HPLC–ECD and chemometrics. *Food Chemistry*, 194, 167-174.

Table 1. Colour, total phenolic content and antioxidant activity of monofloral honeys grouped and mediated by botanical origin.

HONEY SAMPLES	n	LOVIBOND mm Pfund	TPC mg GAE/kg	ABTS mmol TXE/kg	DPPH-Vis mmol TXE/kg	DPPH-EPR mmol TXE/kg
BLACK LOCUST (L)	28	8 ± 7 ^E	197 ± 33 ^E	1.8 ± 0.6 ^D	0.26 ± 0.06 ^F	0.22 ± 0.06 ^G
CITRUS (C)	7	11 ± 5 ^E	157 ± 7 ^E	1.2 ± 0.3 ^D	0.16 ± 0.02 ^F	0.22 ± 0.09 ^{F, G}
SUNFLOWER (C)	33	51 ± 7 ^D	303 ± 50 ^D	4.7 ± 0.7 ^C	0.49 ± 0.11 ^{E, F}	0.44 ± 0.11 ^{E, F}
CORIANDER (C)	7	54 ± 11 ^D	404 ± 64 ^C	4.3 ± 0.4 ^C	0.63 ± 0.13 ^{E, F}	0.59 ± 0.13 ^{E, F}
EUCALYPTUS (C)	7	54 ± 10 ^D	379 ± 70 ^{C, D}	5.8 ± 0.3 ^B	0.79 ± 0.18 ^{D, E}	0.76 ± 0.18 ^{D, E}
STRAWBERRY TREE (C)	7	70 ± 7 ^C	850 ± 133 ^A	7.5 ± 1.0 ^A	4.6 ± 0.9 ^A	4.3 ± 0.6 ^A
CHESTNUT (L)	10	72 ± 7 ^C	618 ± 49 ^B	4.4 ± 0.8 ^C	1.2 ± 0.3 ^{C, D}	1.1 ± 0.3 ^{C, D}
FIR HONEYDEW (L)	7	84 ± 5 ^B	596 ± 42 ^B	5.1 ± 0.2 ^{B, C}	1.5 ± 0.2 ^C	1.4 ± 0.3 ^C
HONEYDEW (L)	11	98 ± 10 ^A	801 ± 131 ^A	6.0 ± 0.2 ^B	2.2 ± 0.7 ^B	1.8 ± 0.5 ^B

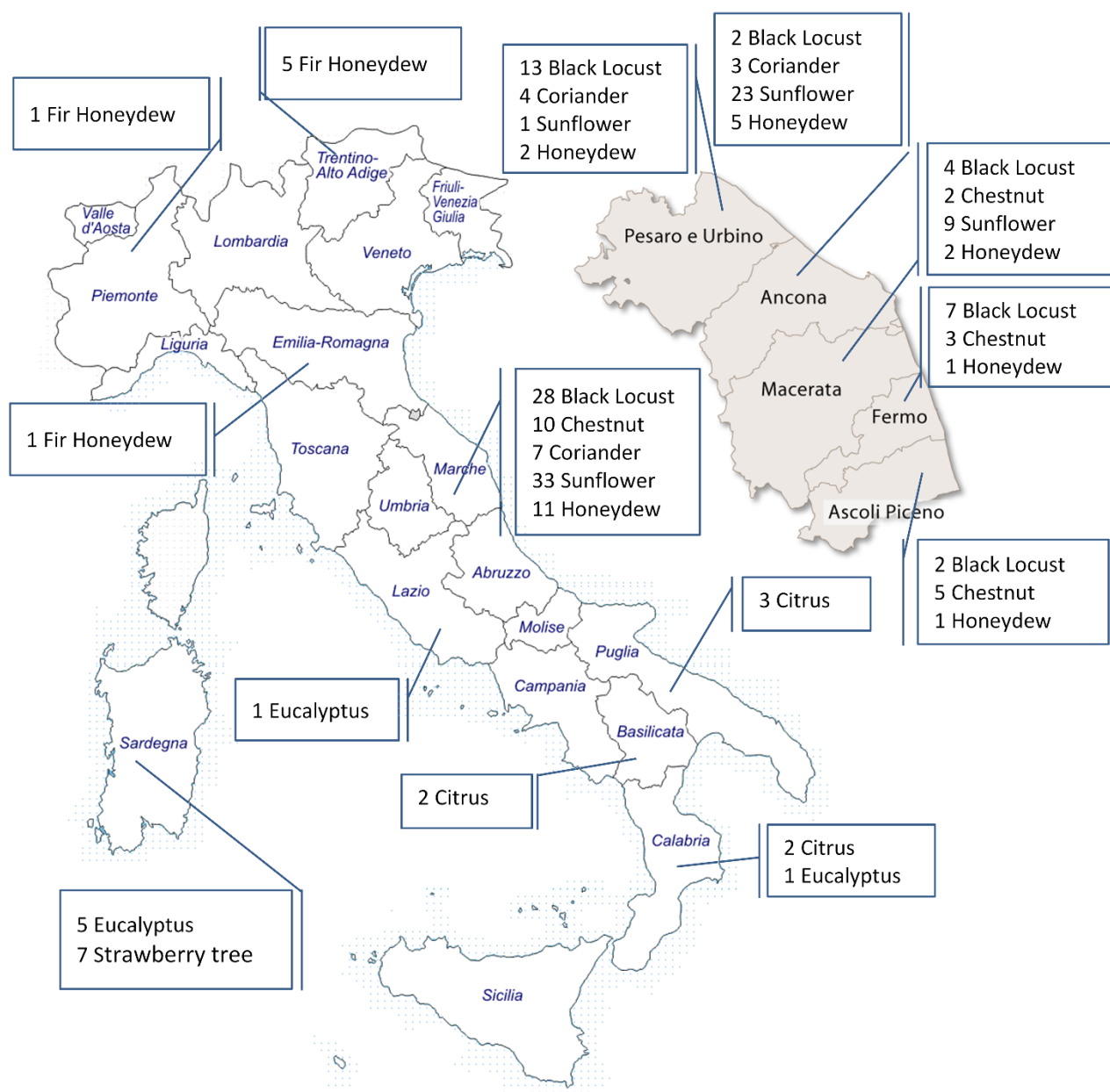
n: number of samples; Lovibond: colour; TPC: total phenol content; ABTS: ABTS test; DPPH-VIS: DPPH test monitored by absorbance; DPPH-EPR: DPPH test monitored by EPR; L: liquid; C: crystallized; GAE: gallic acid equivalents; TXE: Trolox equivalents. Values are expressed as means ± SD. In the same column, different letters indicate significant difference at $p \leq 0.05$.

Table 2: Principal Component Analysis. Eigenvalues, explained and cumulative variance, loadings of the variables for the first three principal components (PC).

Variance explained	PC1	PC2	PC3
Eigenvalues	8.716	1.467	1.096
% of variance	72.637	12.228	9.136
Cumulative %	72.637	84.865	94.001
Factor loading			
TPC	0.894	-0.026	0.432
ABTS	0.793	-0.506	0.170
DPPH-EPR	0.621	-0.279	0.702
Lovibond	0.947	-0.146	0.009
Crystallization	0.068	0.896	0.276
λ_{350}	0.843	0.388	-0.033
λ_{360}	0.932	0.282	-0.027
λ_{365}	0.970	0.196	-0.032
λ_{385}	0.985	0.117	-0.055
λ_{445}	0.959	-0.067	-0.212
λ_{490}	0.882	-0.143	-0.383
λ_{645}	0.901	-0.034	-0.339

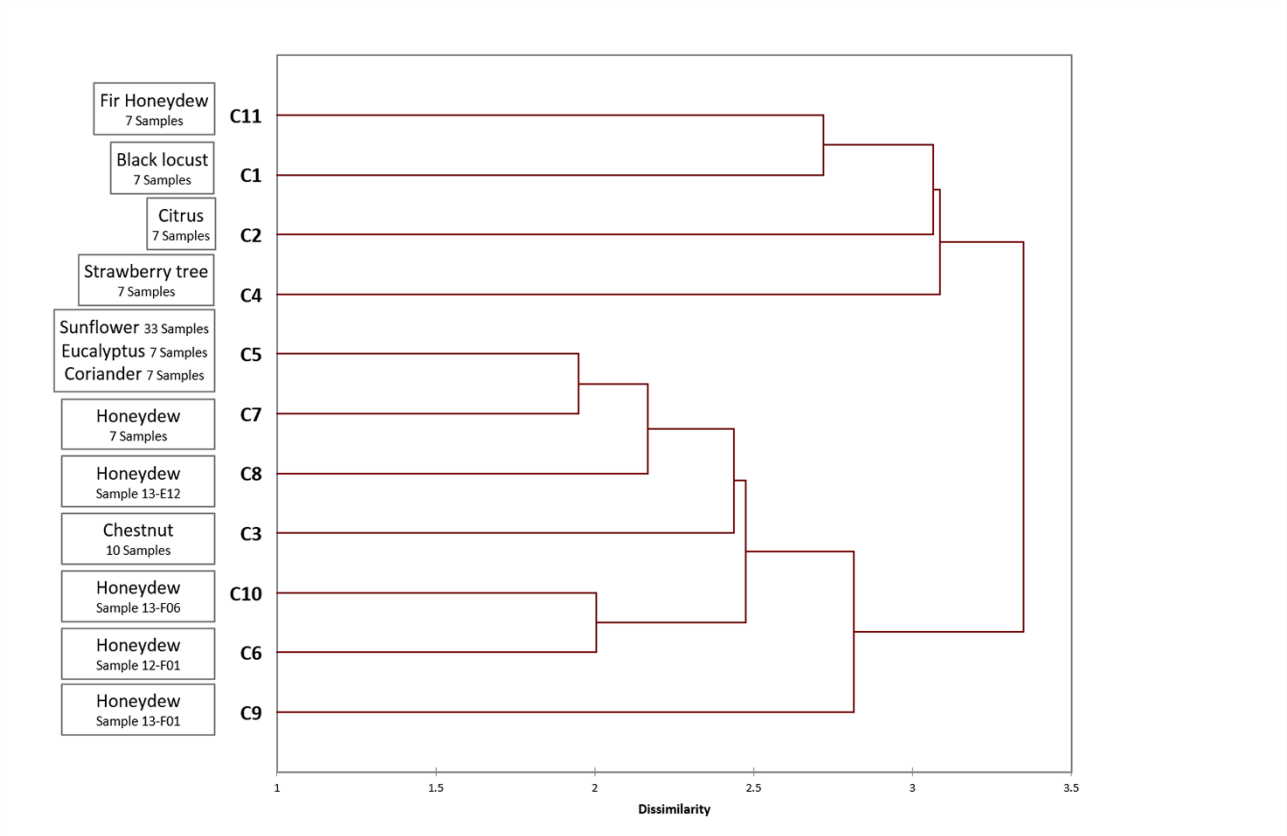
TPC: total phenol contents; ABTS: ABTS test; DPPH-EPR: DPPH test monitored by EPR; Lovibond: colour; Crystallization: liquid (1) crystallized (0).

Figure 1.



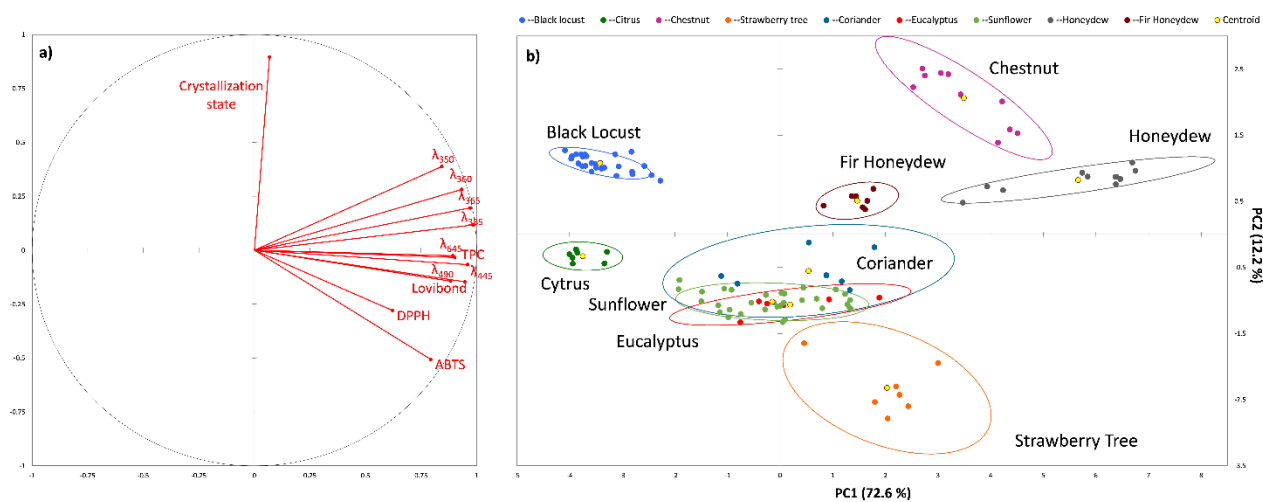
Geographical origin of the 117 monofloral honey samples [28 Black Locust (*Robinia pseudoacacia* L.), 7 Citrus (*Citrus* spp.), 33 Sunflower (*Helianthus annuus* L.), 7 Coriander (*Coriandrum sativum* L.), 7 Eucalyptus (*Eucalyptus* spp.), 7 Strawberry tree (*Arbutus unedo* L.), 10 Chestnut (*Castanea sativa* Mill.), 7 Fir honeydew (*Fir*: *Abies alba* Mill. and *Picea abies* L.), and 11 Honeydew (Forest honeydew, *Insect*: *Metcalfa pruinosa* (Say)] collected in the different Italian regions (Marche, Basilicata, Calabria, Emilia Romagna, Piemonte, Puglia, Sardegna and Trentino Alto Adige).

Figure 2.



Dendrogram obtained by Cluster analysis performed on the whole data set incorporating 117 honey samples and 13 variables (FOLIN, ABTS, DPPH-Vis, DPPH-EPR, Lovibond, λ_{350} , λ_{360} , λ_{365} , λ_{385} , λ_{445} , λ_{490} , λ_{645} and crystallization state), and using the single linkage algorithm (Euclidean distance).

Figure 3.



Principal component analysis. Loading plot (a) and score plot (b) of the first two components. TPC: total phenol contents; ABTS: ABTS test; DPPH-EPR: DPPH test monitored by EPR; Lovibond: colour; Crystallization: liquid (1) crystallized (0).

SUPPLEMENTARY

Table S1. Crystallization state, colour, total phenolic content and antioxidant activity of monofloral honeys samples. Honeys are grouped by botanical origin and harvesting season. Samples are numbered as (year-code).

HONEY SAMPLES	LOVIBOND mm Pfund	TPC mg GAE/kg	ABTS mmol TXE/kg	DPPH-Vis mmol TXE/kg	DPPH-EPR mmol TXE/kg	Cryst. State C=0, L= 1
BLACK LOCUST						
12-B03	5	173 ± 10	1.3 ± 0.2	0.15 ± 0.05	0.11 ± 0.05	1
12-E07	5	196 ± 4	1.3 ± 0.2	0.18 ± 0.05	0.21 ± 0.01	1
12-F02	10	246 ± 11	2.3 ± 0.3	0.27 ± 0.05	0.26 ± 0.00	1
12-F03	30	268 ± 8	3.1 ± 0.1	0.24 ± 0.04	0.23 ± 0.06	1
12-G08	5	174 ± 6	1.2 ± 0.1	0.17 ± 0.05	0.15 ± 0.03	1
12-G10	5	212 ± 8	1.5 ± 0.1	0.29 ± 0.05	0.33 ± 0.12	1
12-H01	5	151 ± 20	1.1 ± 0.1	0.30 ± 0.09	0.15 ± 0.11	1
12-I06	10	222 ± 8	1.6 ± 0.1	0.38 ± 0.05	0.43 ± 0.14	1
12-I11	5	161 ± 13	1.2 ± 0.2	0.19 ± 0.02	0.20 ± 0.07	1
13-A01	5	192 ± 13	2.2 ± 0.2	0.29 ± 0.02	0.23 ± 0.04	1
13-A02	25	274 ± 15	2.7 ± 0.3	0.37 ± 0.03	0.26 ± 0.04	1
13-A03	5	174 ± 15	1.8 ± 0.2	0.26 ± 0.05	0.18 ± 0.01	1
13-A04	15	211 ± 6	2.6 ± 0.3	0.33 ± 0.03	0.23 ± 0.02	1
13-A05	5	177 ± 11	1.7 ± 0.2	0.29 ± 0.04	0.20 ± 0.02	1
13-A06	5	188 ± 19	2.3 ± 0.1	0.26 ± 0.06	0.19 ± 0.01	1
13-A07	5	198 ± 15	2.0 ± 0.2	0.28 ± 0.02	0.22 ± 0.02	1
13-A08	10	222 ± 9	2.7 ± 0.3	0.34 ± 0.06	0.29 ± 0.02	1
13-A09	5	167 ± 8	1.2 ± 0.1	0.25 ± 0.03	0.20 ± 0.01	1
13-A10	5	201 ± 9	2.2 ± 0.3	0.31 ± 0.05	0.28 ± 0.03	1
13-A11	0	174 ± 13	1.2 ± 0.1	0.25 ± 0.05	0.23 ± 0.04	1
13-A12	5	163 ± 5	1.7 ± 0.2	0.23 ± 0.04	0.20 ± 0.01	1
13-B01	5	184 ± 10	1.2 ± 0.1	0.25 ± 0.05	0.27 ± 0.17	1
13-B02	10	214 ± 22	2.1 ± 0.2	0.26 ± 0.04	0.21 ± 0.03	1
13-B03	10	231 ± 20	2.1 ± 0.1	0.29 ± 0.03	0.25 ± 0.02	1
13-B04	15	242 ± 21	2.8 ± 0.2	0.30 ± 0.02	0.24 ± 0.06	1
13-B05	0	157 ± 12	0.8 ± 0.1	0.17 ± 0.03	0.21 ± 0.17	1
13-B06	0	171 ± 14	1.2 ± 0.1	0.19 ± 0.03	0.15 ± 0.02	1
13-B07	5	181 ± 13	1.9 ± 0.2	0.17 ± 0.03	0.17 ± 0.04	1
CITRUS						
14-A01	15	159 ± 5	1.8 ± 0.2	0.17 ± 0.03	0.32 ± 0.12	0
14-A02	20	163 ± 7	1.0 ± 0.1	0.17 ± 0.02	0.33 ± 0.15	0
14-A03	10	153 ± 12	1.7 ± 0.1	0.16 ± 0.02	0.15 ± 0.05	0
14-A04	5	147 ± 9	1.0 ± 0.1	0.18 ± 0.02	0.26 ± 0.10	0
14-A05	10	154 ± 11	1.1 ± 0.1	0.18 ± 0.02	0.17 ± 0.10	0
14-A06	10	153 ± 7	1.2 ± 0.2	0.13 ± 0.02	0.16 ± 0.18	0
14-A07	10	167 ± 5	1.0 ± 0.1	0.15 ± 0.02	0.12 ± 0.09	0
SUNFLOWER						
12-A02	45	253 ± 18	3.9 ± 0.4	0.31 ± 0.11	0.41 ± 0.00	0
12-A03	55	320 ± 21	4.6 ± 0.4	0.37 ± 0.12	0.52 ± 0.06	0
12-A04	45	250 ± 19	4.0 ± 0.5	0.33 ± 0.06	0.44 ± 0.00	0
12-A09	60	399 ± 22	5.2 ± 0.2	0.58 ± 0.07	0.65 ± 0.05	0
12-C02	50	383 ± 21	4.8 ± 0.2	0.59 ± 0.04	0.57 ± 0.02	0
12-D03	50	361 ± 35	5.6 ± 0.3	0.60 ± 0.04	0.51 ± 0.09	0
12-D05	50	334 ± 37	5.3 ± 0.1	0.48 ± 0.05	0.52 ± 0.05	0
12-E11	55	367 ± 20	4.0 ± 0.2	0.51 ± 0.07	0.45 ± 0.05	0
12-F12	55	266 ± 10	4.3 ± 0.3	0.31 ± 0.09	0.34 ± 0.01	0
12-H06	50	345 ± 19	4.3 ± 0.2	0.62 ± 0.10	0.60 ± 0.16	0
12-I08	55	310 ± 8	3.9 ± 0.2	0.42 ± 0.11	0.55 ± 0.06	0
13-C12	45	263 ± 14	4.2 ± 0.5	0.45 ± 0.04	0.38 ± 0.02	0
13-D01	45	259 ± 20	4.0 ± 0.3	0.40 ± 0.04	0.34 ± 0.03	0
13-D02	60	300 ± 20	4.7 ± 0.3	0.54 ± 0.03	0.48 ± 0.03	0
13-D03	55	290 ± 22	5.0 ± 0.4	0.47 ± 0.03	0.53 ± 0.08	0
13-D04	60	302 ± 24	4.6 ± 0.4	0.54 ± 0.03	0.51 ± 0.01	0
13-D05	55	339 ± 23	5.1 ± 0.4	0.53 ± 0.02	0.50 ± 0.02	0

13-D06	50	369 ± 23	5.5 ± 0.5	0.62 ± 0.02	0.57 ± 0.04	0
13-D07	45	292 ± 17	5.0 ± 0.3	0.43 ± 0.02	0.36 ± 0.01	0
13-D08	45	247 ± 28	3.6 ± 0.2	0.35 ± 0.02	0.33 ± 0.03	0
13-D09	45	261 ± 24	4.4 ± 0.4	0.37 ± 0.02	0.35 ± 0.03	0
13-D10	55	296 ± 19	5.6 ± 0.4	0.49 ± 0.02	0.48 ± 0.02	0
13-D11	55	321 ± 23	6.0 ± 0.1	0.52 ± 0.02	0.47 ± 0.04	0
13-D12	45	242 ± 16	3.7 ± 0.2	0.35 ± 0.02	0.36 ± 0.02	0
13-E01	45	277 ± 35	5.0 ± 0.2	0.59 ± 0.04	0.29 ± 0.02	0
13-E02	45	230 ± 8	4.6 ± 0.2	0.48 ± 0.19	0.27 ± 0.02	0
13-E03	50	260 ± 26	4.4 ± 0.2	0.51 ± 0.08	0.28 ± 0.01	0
13-E04	45	304 ± 23	5.5 ± 0.2	0.53 ± 0.06	0.36 ± 0.01	0
13-E05	35	237 ± 16	3.6 ± 0.2	0.43 ± 0.03	0.29 ± 0.02	0
13-E06	55	311 ± 20	5.0 ± 0.3	0.60 ± 0.06	0.48 ± 0.03	0
13-E07	65	368 ± 19	5.4 ± 0.3	0.70 ± 0.07	0.58 ± 0.05	0
13-E08	65	394 ± 24	6.0 ± 0.0	0.73 ± 0.08	0.54 ± 0.03	0
13-E09	50	247 ± 17	5.3 ± 0.2	0.45 ± 0.10	0.23 ± 0.01	0
CORIANDER						
12-L03	50	455 ± 8	4.1 ± 0.2	0.54 ± 0.04	0.56 ± 0.00	0
13-C05	65	439 ± 35	5.2 ± 0.6	0.80 ± 0.06	0.69 ± 0.03	0
13-C06	50	341 ± 26	4.3 ± 0.5	0.56 ± 0.05	0.53 ± 0.01	0
13-C07	45	367 ± 23	4.3 ± 0.5	0.55 ± 0.04	0.50 ± 0.04	0
13-C08	55	450 ± 23	4.3 ± 0.5	0.67 ± 0.05	0.68 ± 0.05	0
13-C09	70	468 ± 23	4.2 ± 0.4	0.82 ± 0.04	0.76 ± 0.04	0
13-C10	40	307 ± 24	3.7 ± 0.5	0.50 ± 0.05	0.40 ± 0.03	0
EUCALYPTUS						
14-C01	65	424 ± 24	5.8 ± 0.4	0.96 ± 0.03	0.90 ± 0.03	0
14-C02	50	353 ± 25	5.6 ± 0.5	0.72 ± 0.01	0.68 ± 0.03	0
14-C03	45	352 ± 26	5.8 ± 0.4	0.70 ± 0.01	0.58 ± 0.05	0
14-C04	45	336 ± 16	5.4 ± 0.6	0.71 ± 0.02	0.57 ± 0.04	0
14-C05	70	516 ± 18	6.3 ± 0.1	1.11 ± 0.02	1.03 ± 0.06	0
14-C06	55	363 ± 22	5.6 ± 0.5	0.74 ± 0.01	0.89 ± 0.21	0
14-C07	45	307 ± 18	6.2 ± 0.0	0.59 ± 0.01	0.68 ± 0.09	0
STRAWBERRY TREE						
14-B02	70	839 ± 30	7.3 ± 0.1	4.52 ± 0.14	4.00 ± 0.24	0
14-B03	65	907 ± 24	7.9 ± 0.1	5.21 ± 0.24	4.69 ± 0.15	0
14-B04	65	870 ± 36	7.8 ± 0.2	4.97 ± 0.20	4.51 ± 0.26	0
14-B05	70	959 ± 35	8.3 ± 0.2	5.30 ± 0.20	4.85 ± 0.26	0
14-B06	70	563 ± 29	5.3 ± 0.1	2.85 ± 0.11	3.12 ± 0.05	0
14-B07	85	876 ± 24	7.3 ± 0.2	4.07 ± 0.24	3.91 ± 0.37	0
14-B08	65	937 ± 26	8.4 ± 0.2	5.36 ± 0.21	4.75 ± 0.19	0
CHESTNUT						
12-F04	70	622 ± 17	4.8 ± 0.3	1.19 ± 0.12	1.07 ± 0.05	1
12-G03	70	633 ± 33	3.8 ± 0.2	1.08 ± 0.09	0.95 ± 0.02	1
12-G11	55	594 ± 38	3.6 ± 0.1	0.91 ± 0.08	0.81 ± 0.03	1
12-H09	70	550 ± 28	3.5 ± 0.4	0.93 ± 0.08	1.05 ± 0.10	1
12-I01	70	712 ± 41	4.0 ± 0.1	0.99 ± 0.10	1.10 ± 0.09	1
12-I03	70	618 ± 42	4.0 ± 0.4	0.91 ± 0.10	0.99 ± 0.10	1
13-B11	80	671 ± 18	5.4 ± 0.3	1.79 ± 0.06	1.64 ± 0.09	1
13-B12	80	637 ± 29	5.7 ± 0.4	1.69 ± 0.07	1.46 ± 0.11	1
13-C01	70	555 ± 52	4.5 ± 0.3	1.01 ± 0.06	0.94 ± 0.04	1
13-C02	80	593 ± 31	5.1 ± 0.4	1.48 ± 0.06	1.33 ± 0.09	1
FIR HONEYDEW						
14-D01	75	576 ± 32	5.0 ± 0.1	1.56 ± 0.07	1.41 ± 0.02	1
14-D02	80	633 ± 30	5.1 ± 0.1	1.33 ± 0.04	1.18 ± 0.03	1
14-D03	85	540 ± 26	5.2 ± 0.2	1.26 ± 0.03	1.05 ± 0.11	1
14-D04	90	580 ± 25	5.4 ± 0.1	1.46 ± 0.07	1.47 ± 0.24	1
14-D05	85	670 ± 29	5.2 ± 0.1	1.77 ± 0.06	1.98 ± 0.33	1
14-D06	85	581 ± 28	4.9 ± 0.1	1.67 ± 0.08	1.48 ± 0.07	1
14-E12	90	591 ± 27	5.0 ± 0.2	1.48 ± 0.11	1.47 ± 0.03	1
HONEYDEW						
12-F01	95	1039 ± 39	6.3 ± 0.0	3.48 ± 0.06	2.69 ± 0.70	1
12-H02	95	836 ± 18	5.6 ± 0.2	1.63 ± 0.04	1.40 ± 0.08	1
12-I02	90	893 ± 36	6.3 ± 0.0	2.40 ± 0.12	1.79 ± 0.14	1
13-E11	100	741 ± 34	6.0 ± 0.1	1.79 ± 0.05	1.50 ± 0.11	1

13-E12	100	864 ± 31	6.1 ± 0.0	3.05 ± 0.03	2.62 ± 0.20	1
13-F01	95	692 ± 40	6.0 ± 0.1	1.92 ± 0.06	1.58 ± 0.22	1
13-F02	114	821 ± 31	6.0 ± 0.1	2.07 ± 0.25	1.78 ± 0.14	1
13-F03	114	775 ± 38	6.0 ± 0.1	1.65 ± 0.04	1.47 ± 0.13	1
13-F04	85	647 ± 44	6.0 ± 0.1	1.61 ± 0.15	1.45 ± 0.11	1
13-F05	85	583 ± 42	5.9 ± 0.1	1.43 ± 0.10	1.25 ± 0.11	1
13-F06	100	919 ± 57	6.1 ± 0.0	3.34 ± 0.06	2.61 ± 0.17	1

Table S2. Discriminant Analysis. Unidimensional test of equality of the means of the classes of DA performed on the whole data set incorporating 117 honey samples classified into 9 groups and 71 variables (λ_{350} to λ_{700} , every 5 nm).

Variable	Lambda	F	DF1	DF2	p-value
350	0.050	255.121	8	108	< 0.0001
360	0.067	189.216	8	108	< 0.0001
365	0.071	175.560	8	108	< 0.0001
385	0.082	151.783	8	108	< 0.0001
445	0.133	88.301	8	108	< 0.0001
490	0.170	65.689	8	108	< 0.0001
645	0.193	56.547	8	108	< 0.0001

Table S3. Cluster Analysis. Descriptive statistics for the Cluster analysis performed on the whole data set incorporating 117 honey samples and 13 variables (FOLIN, ABTS, DPPH-Vis, DPPH-EPR, Lovibond, λ_{350} , λ_{360} , λ_{365} , λ_{385} , λ_{445} , λ_{490} , λ_{645} and crystallization state), and using the single linkage algorithm (Euclidean distance).

Cluster	Number of objects	Sum of weights	Within-class variance	Minimum distance to the centroid	Mean distance to the centroid	Maximum distance to the centroid	Objects
1	28	28	0.351	0.185	0.521	1.304	Black locust
2	7	7	0.170	0.190	0.355	0.595	Citrus
3	10	10	1.636	0.385	1.171	1.586	Chestnut
4	7	7	2.067	0.530	1.157	2.623	Strawberry tree
5	47	47	1.775	0.281	1.228	2.308	Coriander, Eucalyptus, Sunflower
6	1	1	0.000	0.000	0.000	0.000	Honeydew
7	7	7	2.204	0.289	1.249	2.328	Honeydew
8	1	1	0.000	0.000	0.000	0.000	Honeydew
9	1	1	0.000	0.000	0.000	0.000	Honeydew
10	1	1	0.000	0.000	0.000	0.000	Honeydew
11	7	7	0.412	0.355	0.568	0.822	Fir Honeydew

Figure S1. Principal component analysis: PC2 vs. PC3. Score plot of the second and the third components.

