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ORIGINAL PAPER

Floral authentication of some monofloral honeys based on volatile composition and physicochemical parameters

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Abstract

Twenty four honey samples of eight distinct botanical origins obtained from different regions of Turkey were analyzed in this study. Various physicochemical (glucose and fructose content, color, diastase activity, electrical conductivity, optical rotation, moisture and proline content), biochemical (total phenolic content, total flavonoid content, FRAP antioxidant capacity and DPPH radical scavenging activity) and volatile analytical methods were used for floral authentication of the honeys. Identification of 103 volatile compounds from different chemical classes was carried out using SPME/GC/MS. The availability of physicochemical and volatile identification methods, instead of melissopalynology which is dependent on the researcher's ability and judgment regarding honey classification, was investigated. The results were statistically processed, and principal component analysis (PCA) was applied. The first two principal components explained 98.10% of the total variance, and the PCA produced eight different groups rhododendron, chestnut, lavandula, astragalus, chaste tree, polyfloral, oak, and pine honeys, each corresponding to a distinct botanical origin. Polyfloral honeys formed a cluster, although those samples were rather widespread. The studied honeys exhibited specific volatile compounds and biochemical properties which may be capable of use as floral markers.

Keywords Monofloral · Honey · Marker · Authentication · Aroma · Volatiles

Introduction

The prominent position occupied by bee products worldwide has resulted in significant interest and research into honey, and its bioactive properties and characterization. The bioactive characteristics of honey differ due to the differences in botanical and geographical origin, together with climatic, processing and storage conditions. In terms of botanical origin, honey is classified as monofloral or multifloral, depending on whether plants arise predominantly from a single or several species, respectively. Establishing the monoflorality of honey is also of economic importance, since a honey with

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nectar and pollen belonging predominantly to a specific species may have a higher selling price, allowing beekeepers to generate higher incomes. Monofloral honeys differ from one another in terms of their volatile compositions, which in turn have a major effect on the sensory characteristics of each type of honey [1]. The development of fast, reliable and cost-effective methodologies for the discrimination and classification of honeys based on their botanical origins is, therefore, of considerable importance [2]. A broad range of analytical platforms, such as gas nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (IR), chromatography/mass spectrometry (GC/MS), front face fluorescence spectroscopy, atomic emission spectroscopy (AES), and inductively coupled plasma atomic emission spectrometry (ICP-AES), has been used for the chemical characterization of honeys and their classification. The GC/MS finger printing method has been proved to be a powerful platform for discriminating between honey samples [3].

Specific volatile compounds have been identified in a number of monofloral honeys, and these are regarded as markers by some researchers [4–8]. The aroma compounds detected in honey are generally reported to be caused by the plant constituents, the transformation of those constituents and direct generation of aroma compounds by the honeybee, the generation of aroma compounds by thermal honey processing, and flavor contaminants in the honey [4]. Volatile compounds, which primarily account for food aroma and flavor, are present in honey at very low concentrations in the form of complex mixtures of different chemical classes, including monoterpenes, norisoprenoids, sesquiterpenes, benzenoids, alcohols, esters, ketones and aldehydes [1, 9].

Due to the limitations associated with traditional methods, novel methodologies for the determination of botanical origin are emerging to replace melissopalynology. Analysis of the key aroma compounds of honey has become important for assessing botanical origin. This research prioritizing the study of the biochemical characteristics, microscopic analysis of honey sediment, volatile compounds of different honeys including monofloral, polyfloral, and honeydew honeys collected from Turkey was, therefore, performed in detail. Turkey is one of the richest regions of the world in terms of the production and variety of honey, due to features such as its geographical position, climatic conditions, and three seasons of the year being suited to honey production [10].

The principal aim of the present research was to comprehensively characterize rhododendron, chestnut, lavandula, astragalus, chaste tree, oak, pine, and polyfloral Turkish honeys in terms of their physicochemical parameters (moisture content, conductivity, color, diastase, sugars, proline, total phenolic content, flavonoid content, and antioxidant activity) and volatile compounds through the application of multivariate analysis, with the ultimate objective of achieving better differentiation.

Materials and methods

Honey samples

Monofloral pure blossom honeys (rhododendron (*Rhododendron ponticum* L.), chestnut (*Castanea sativa* Mill.) lavandula, (*Lavandula stoechas* L.), astragalus (*Astragalus microcephalus* Willd.), chaste tree *(Vitex agnus castus*), polyfloraland honeydew honeys oak (*Quercus robur* L.), and pine (*Pinus* L.)) were obtained from experienced beekeepers from different areas of Turkey in 2018–2019 for use in the study. Honeys were stored in the dark at + 4 °C until analysis.

Physicochemical analysis

Melissopalynological studies were performed as described previously [11]. Moisture content was determined using the refractometric method with the refractive index table as shown in AOAC 969.38 [12]. Optical activity and electrical conductivity were measured using a polarimeter (Beta PPP7 Optical Activity, Cambridge, UK) and a conductometer (WTW inoLab Cond/720, Germany), respectively, as described by Junk and Pancoast [13]. The colors of honeys were read using a colorimeter (Konica Minolta CR5, Osaka, Japan) and expressed as *L*, a*, b** values. Proline contents were determined according to the IHC method [14]. Diastase activities of honey samples were performed using the Phadebas method [15].

Determination of total phenolic content, flavonoid content, and antioxidant activity

Total phenolic contents were determined using the Folin–Ciocalteu method as reported by Hayaloglu and Demir [16]. The obtained results were expressed as mg GAE per 100 g of honey (GAE—gallic acid equivalent). The spectrophotometric method has been performed to measure the total flavonoid contents of the studied samples as described previously by Kocabey et al. [17] using quercetin as standard.

Ferric tripyridyltriazine [Fe (III)-TPTZ] complex was used to measure total antioxidant capacity [16]. FRAP reagent was prepared mixing 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ dissolved in 40 mM HCl, and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solutions. A 100 µL of the sample was mixed with 3 mL of freshly prepared FRAP reagent, the mixture was incubated at 37 ºC. The absorbance value of this mixture was measured at 595 nm against reagent blank containing distilled water at the end of the 4 min of incubation time. The tubes were also prepared with

15.625–500 µM of Trolox to construct a reference curve ($R^2 = 0.999$). The FRAP values being calculated as µM Trolox equivalent per gram of honey.

The scavenging of DPPH radicals was performed using the method given by Kocabey et al. [17]. The assay was based on the mechanism that the radical is purple-colored, and this dark color of DPPH turns to yellow with the existence of the antioxidant compounds. The change in absorbance was measured at 517 nm using a spectrophotometer.

Sugar analysis

Sugar analysis was performed using the procedure described by Ozturk et al. [18]. Honey sugars were monitored using HPLC (Elite LaChrom, Hitachi, Japan) equipped with a refractive index detector (RID) wand a reverse-phase amide column (200/4.6 Detector-Nucleosil 100–5 NH2). The constructed calibration curves had R^2 between 0.994 and 1.000.

Volatile compounds analysis

A 50/30 µm divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA) was used to extract headspace of volatiles from honey samples. The separations were performed using a ZB-WAX plus capillary column (60 m × 0.25 mm) with thickness of 0.25 µm, helium as the carrier gas (purity 99.999%) at a 1.0 mL/min of flow rate. The oven temperature was held at 40 °C for 2 min, and increased to 250 °C at 8 °C/ min (6-min hold). The fiber was conditioned in accordance with the manufacturers' instructions. A mixture of 2 g of honey in 2 mL of distilled water and 0.20 g NaCl (Merck, Darmstadt, Germany) was inserted in a 15 mL crimp-cap vial. The vial sealed with septa (silicone/ PTFE). The sample vial was kept at 45 °C in a temperature-controlled water bath under constant stirring at 600 rpm during the extraction process. The extraction was performed using the following conditions: extraction temperature (40 °C), equilibration time (30 min), sampling time (30 min), vial size (15 mL), and fiber coating film including CAR/PDMS, PDMS, DVB/CAR/PDMS. Analyzed samples were prepared daily just before HS-SPME–GC/MS analysis. Measurements were run in duplicate. Compounds were searched using the identification program of Wiley 7 NIST05 mass spectral library. A mixture of n-alkanes series (C8–C20) (Supelco, Bellefonte, PA, USA) was used to calculate retention indices. The results were represented as percentages of the total chromatogram area [19].

Statistical analysis

The results were expressed as mean values and standard deviations (mean ± SD) for five replicates. Data were tested using SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The Kruskal–Wallis and Mann–Whitney U tests and non-parametric Pearson correlation analysis were applied during statistical analyses. p values < 0.05 were accepted as the statistically significant. Principal component analysis (PCA) of the chemical composition and volatile compound data was performed on SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). BioVinci version 3.0.9 (developed by BioTuring Inc., San Diego, CA, USA) visualization tools were also used.

Results and discussion

Physicochemical characteristics and bioactive properties

The most frequent pollen types present in honey samples, at frequencies > 45%, are known as dominant pollen. Honey is classified as monofloral when the pollen frequency of one specific plant exceeds 45%. Table 1 shows the dominant pollen families in the honey samples investigated in the present study. Overall optical rotation depends on the content of various saccharides in honey, and is the sum of the rotations of individual saccharide compounds present in a particular sample [20]. The botanical origins of pine and oak honeys were determined using palynological tests, together with their optic rotations. With the exception of pine and oak honeys, the honeys in this study all exhibit negative optical activity.

The moisture content of honey is an important factor influencing its stability and crystallization characteristics during storage. Its moisture content varies depending on the environmental conditions and manipulation by beekeepers during harvesting [21]. The moisture contents of all the samples in the present study were below 20% (Table 2), the highest moisture level for honey permitted by the Turkish

Table 1 Pollen types in the honey samples

Samples	Dominant pollen	
	Type	%
Rhododendron	*Rhododendron ponticum* L	69.3 ± 6.08
Chestnut	*Castanea sativa* Mill	95.66 ± 1.15
Lavandula	*Lavandula stoechas* L	58.76 ± 3.05
Astragalus	*Astragalus microcephalus* Willd	68.88 ± 2.92
Chaste tree	*Vitex agnus castus*	54.75 ± 4.10
Oak	*Quercus robur* L	No pollen
Pine	*Pinus L*	No pollen
Polyfloral		Less than 30% of all pollen types

Data represent the mean values and standard deviations

Table 2 Physicochemical parameters of honeys according to botanical origin

Sample	Moisture (%)	Conductivity (mS/cm)	Proline (mg/kg)	Diastase (D.U)	Optical rotation [α]20	L*	a*	b*	F/G	F+G	Total phenolic (mg GAE/100 g)	Total flavonoid (mg QE/100 g)	FRAP µmolTrolox/g	DPPH SC_{50} (mg/mL)
Rhododendron	17.13 ± 0.58	0.64 ± 0.12	868.67 ± 42.57	6.66 ± 0.94	− 2.47 ± 0.86	78.63 ± 0.78	6.89 ± 0.53	70.28 ± 0.56	1.33 ± 0.02	62.75 ± 4.19	49.53 ± 8.10	0.78 ± 0.12	1.27 ± 0.21	35.67 ± 8.57
Chestnut	19.33 ± 0.99	1.54 ± 0.30	847.77 ± 46.36	7.19 ± 0.61	− 2.72 ± 1.66	52.93 ± 4.60	36.09 ± 3.10	87.67 ± 5.56	2.02 ± 0.63	53.42 ± 4.07	70.80 ± 22.38	6.50 ± 1.73	5.19 ± 0.93	22.65 ± 2.28
Lavandula	18.40 ± 0.69	0.25 ± 0.04	620.58 ± 82.77	6.73 ± 1.85	− 2.37 ± 0.43	62.97 ± 2.29	15.78 ± 0.01	72.05 ± 1.40	1.77 ± 0.57	62.99 ± 12.57	67.21 ± 12.48	1.83 ± 0.88	0.54 ± 0.23	110.57 ± 13.10
Astragalus	17.00 ± 0.59	0.40 ± 0.05	805.83 ± 23.88	7.42 ± 0.42	− 1.70 ± 0.21	74.48 ± 1.23	11.19 ± 0.56	72.04 ± 1.36	1.47 ± 0.46	64.21 ± 3.76	59.53 ± 17.56	0.67 ± 0.21	0.89 ± 0.18	72.79 ± 10.72
Chaste tree	17.34 ± 0.10	0.50 ± 0.42	765.50 ± 139.23	13.07 ± 1.68	− 2.32 ± 0.31	44.29 ± 5.91	28.43 ± 0.91	67.51 ± 6.02	1.16 ± 0.01	76.18 ± 1.10	35.93 ± 4.86	0.93 ± 0.04	0.74 ± 0.20	117.97 ± 39.97
Polyfloral	17.20 ± 1.74	1.18 ± 0.70	435.97 ± 199.31	7.07 ± 2.57	− 2.15 ± 0.45	73.82 ± 7.87	12.72 ± 8.74	73.17 ± 14.78	1.34 ± 0.35	51.50 ± 4.11	28.83 ± 3.86	2.01 ± 2.41	0.77 ± 0.50	49.03 ± 14.49
Oak	16.67 ± 1.10	1.15 ± 0.05	414.25 ± 59.21	6.13 ± 1.45	0.47 ± 0.06	43.25 ± 0.15	35.06 ± 0.06	72.48 ± 0.18	1.82 ± 0.10	67.51 ± 2.49	93.87 ± 1.15	3.32 ± 0.49	2.90 ± 0.08	13.20 ± 0.95
Pine	19.16 ± 0.09	1.13 ± 0.17	501.35 ± 171.54	11.13 ± 1.10	0.78 ± 0.03	66.67 ± 9.85	17.74 ± 7.47	81.21 ± 4.29	1.48 ± 0.17	64.43 ± 6.24	60.23 ± 27.51	1.58 ± 1.44	1.53 ± 1.01	50.58 ± 35.55

Food Codex Honey Communiqué [22] and the Codex Standard for Honey [23].

Proline must account for more than 200 mg/kg and at least 66% of the total free amino acids (usually 80–90%) in honey [24]. The highest proline content (868.67 mg/kg) in the present study was observed in rhododendron honey. Honey obtained from sugar-fed honeybee has a lower proline content [20].

Honeydew honey is generally characterized by higher electrical conductivity values than blossom honey. Chestnut honey exhibits the highest electrical conductivity, which is associated with its high mineral content [25]. Diastase activities in this study ranged between 6.13 and 13.07. Light-colored honey also exhibits lower conductivity than darker honey [26]. The L* values of the honey samples ranged from 43 to 78. The dark-colored honeys—chestnut, chaste tree, and oak—exhibited the lowest L* values. The highest redness (+a value) was also observed in chestnut honey. Total phenolic compound, flavonoid content, and ferric reducing antioxidant power values were significantly higher in the dark-colored chestnut and oak honeys. Dark-colored honeys are richer in phenolic components and biological activities [27]. DPPH radical scavenging activity expressed as IC_{50} values (mg/mL) ranged between 13.20 and 117.97. Oak and chestnut honeys exhibited the highest DPPH radical scavenging activities, followed by rhododendron honey. Oak and chestnut honeys have very similar physicochemical properties, making differentiation using routine methods a difficult task [28].

The reducing sugars, fructose and glucose are basic monosaccharides in honey. Fructose and glucose levels found between 51.50 and 76.18%, while the fructose/glucose ratio varied between 1.16 and 2.02. Fructose content was found higher than glucose in all studied samples.

Aroma compounds

The volatile compounds of honeys from various compound classes, such as esters, aldehydes, ketones, alcohols, terpenes, acids and miscellaneous compounds, were identified by GC/MS and are shown in Table 3.

Ethyl acetate and ethyl 4-octanoate were found in all honeys except for lavandula. Ethyl acetate has been previously reported in Spanish lavender and thyme honeys [7]. Silici [29] studied the characterization of volatile compounds from 14 rhododendron honey samples from the Black Sea region of Turkey and identified ethyl benzoate as one of the specific floral origin markers of rhododendron honey. Another compound, ethyl hexanoate, was commonly detected in rhododendron honey. Ethyl isobutanoate and ethyl butanoate were among the predominant ester compounds in chaste tree honeys.

Different aldehydes and ketones were present in all the samples analyzed, including acetone, 4-methyl-3-penten-2-one, 6-methyl-5-hepten-2-one, nonanal, decanal, and benzaldehyde. Linear aldehydes such as nonanal and decanal have been reported as typical honey flavor compounds due to their being identified in numerous honeys from different floral sources [30]. Among these compounds 2-methyl-1-butanal, 3-methyl-1-butanal, 2,3-butanedione, n-hexanal, 2-methyl-2-butenal, and furfural were detected in all honey samples except for oak honey. All samples were found to contain large quantities of furfural. Furan containing-compounds such as furfural can be found naturally in honey, as well as during long-term storage due to sugar degradation or Maillard reaction, and these imparts sweet and fragrant aromas [31]. Significant proportions of nonanal, acetone, decanal, and benzaldehyde were also found in the pine honeys examined. Acetone was more abundant in pine honey compared to the other samples. Previous research has also described acetone as a marker for pine honey [1]. Studies have described nonanal and decanal as being among the compounds that make the greatest contribution to the aroma of Turkish pine honey [32, 33]. Hexanal and heptanal were the most abundant aldehydes in lavandula honey. These have both been described as characteristic of lavender honeys [7]. Hexanal is particularly worthy of note since it appeared in almost all the samples, but at much higher levels in lavender honey.

Six aroma-active alcohol compounds including n-hexanol, cis-3-hexene-1-ol, 2,6-dimethyl-7-octen-2-ol, 2-ethylhexanol, benzyl alcohol, and phenethyl alcohol were identified in all honey samples. Of the numerous alcoholic compounds analyzed, n-hexanol was remarkably high in lavender honey. While 1-pentanol is found only in lavender honey, 6-methyl-5-hepten-2-ol is present in all honeys except for lavender. Hexanol has been determined at high levels in lavandula honey samples [5]. A high level of n-nonyl alcohol was also found in chaste tree and pine honeys. Some studies have suggested that this compound is among the volatile compounds commonly found in eucalyptus [5] and pine honeys [32, 33].

All samples revealed the presence of octanol, except for oak honey, while 2-octanol was found only in pine honeys. Accordingly, the absence and presence of octanol and 2-octanol may be capable of use as markers for differentiating oak and pine honeys, respectively. 1-Octanol has been found in citrus honey and multifloral honey from the southern part of Italy, and *Prosopis caldenia* honey is mainly characterized by the presence of 1-octanol. This compound

Table 3 Volatile compounds of Turkish honeys according to botanical origin

Esters					Honey sample				
RI	Name	Rhododendron	Chestnut	Lavandula	Astragalus	Chaste tree	Polyfloral	Oak	Pine
885	Ethyl acetate	2.67 ± 2.43	3.36 ± 2.66	ND	21.18 ± 18.34	0.64 ± 0.09	1.01 ± 1.41	1.64 ± 0.01	0.25 ± 0.16
965	Ethyl isobutanoate	ND	0.19 ± 0.17	ND	0.41 ± 0.47	0.69 ± 0.25	0.12 ± 0.21	ND	0.32 ± 0.34
1032	Ethyl butanoate	ND	ND	ND	0.17 ± 0.15	0.38 ± 0.06	0.11 ± 0.19	ND	0.69 ± 0.62
1156	3-Methoxy-1-butyl acetate	ND	ND	ND	ND	ND	ND	ND	0.15 ± 0.06
1230	Ethyl hexanoate	1.11 ± 0.16	0.08 ± 0.03	ND	ND	ND	0.51 ± 0.64	ND	ND
1298	Ethyl 2-ethylcaproate	ND	ND	ND	0.20 ± 0.09	0.06 ± 0.01	ND	ND	ND
1401	Ethyl octanate	ND	0.13 ± 0.12	ND	1.35 ± 1.01	0.08 ± 0.08	ND	ND	0.08 ± 0.07
1531	Ethyl 4-octanoate	0.60 ± 0.03	0.24 ± 0.12	ND	0.08 ± 0.02	0.14 ± 0.15	0.31 ± 0.31	0.04 ± 0.07	0.25 ± 0.10
1640	Ethyl decanoate	ND	0.19 ± 0.03	ND	0.11 ± 0.19	0.05 ± 0.04	ND	ND	0.06 ± 0.06
1659	Ethyl 2-nonenoate	0.21 ± 0.02	0.64 ± 0.33	ND	0.45 ± 0.78	ND	1.68 ± 1.45	0.30 ± 0.02	0.01 ± 0.02
1645	Ethyl benzoate	0.23 ± 0.16	1.17 ± 1.20	ND	2.14 ± 1.86	0.13 ± 0.13	0.13 ± 0.11	0.06 ± 0.10	0.03 ± 0.05
1676	Ethyl benzeneacetate	ND	ND	ND	0.26 ± 0.17	0.02 ± 0.04	ND	ND	0.40 ± 0.09
1690	Isopropyl dodecanoate	ND	1.07 ± 0.12	ND	0.26 ± 0.05	0.40 ± 0.19	0.24 ± 0.41	ND	0.63 ± 0.10
1885	Ethyl 3-hydroxyhexanoate	ND	ND	ND	0.07 ± 0.07	0.11 ± 0.03	ND	ND	0.29 ± 0.27
2081	Benzenepropyl acetate	ND	ND	ND	0.02 ± 0.02	0.04 ± 0.04	0.05 ± 0.09	ND	0.21 ± 0.20
Aldehydes and ketones									
811	Acetone	1.36 ± 0.03	2.00 ± 1.34	0.53 ± 0.04	0.11 ± 0.10	2.61 ± 0.65	1.31 ± 1.14	1.11 ± 0.01	6.71 ± 1.68
911	2-Methyl-1-butanal	0.28 ± 0.07	0.42 ± 0.25	0.14 ± 0.02	0.17 ± 0.15	0.58 ± 0.07	0.42 ± 0.27	ND	0.81 ± 0.34
916	3-Methyl-1-butanal	0.59 ± 0.09	1.26 ± 0.70	0.29 ± 0.03	0.41 ± 0.16	1.32 ± 0.16	1.01 ± 0.58	ND	2.12 ± 1.17
964	2,3-Butanedione	0.73 ± 0.03	0.19 ± 0.07	0.24 ± 0.04	0.07 ± 0.12	1.32 ± 0.30	0.74 ± 0.93	ND	0.86 ± 0.79
998	2-Methyl-3-pentanone	ND	ND	ND	0.55 ± 0.07	ND	ND	ND	0.16 ± 0.28
1055	2,3-Pentadione	ND	ND	ND	ND	0.03 ± 0.03	ND	ND	0.03 ± 0.06
1079	n-Hexanal	0.26 ± 0.03	0.42 ± 0.25	12.68 ± 1.02	0.20 ± 0.08	0.11 ± 0.03	0.17 ± 0.16	ND	0.43 ± 0.22
1095	2-Methyl-2-butenal	1.15 ± 0.10	0.74 ± 0.66	0.08 ± 0.01	0.31 ± 0.45	0.44 ± 0.10	0.35 ± 0.30	ND	0.56 ± 0.16
1133	4-Methyl-3-penten-2-one	0.45 ± 0.05	1.21 ± 0.29	0.87 ± 0.10	0.87 ± 0.75	0.13 ± 0.23	0.60 ± 1.04	0.37 ± 0.01	0.40 ± 0.35
1181	2-Heptanone	ND	0.28 ± 0.02	ND	0.20 ± 0.20	0.11 ± 0.11	0.15 ± 0.25	ND	ND
1164	Heptanal	0.14 ± 0.02	0.29 ± 0.21	3.67 ± 0.39	ND	0.18 ± 0.16	0.14 ± 0.13	ND	0.10 ± 0.09
1205	3-Methyl-2-butenal	ND	0,49 ± 0.01	ND	0.37 ± 0.37	0.46 ± 0.48	0.05 ± 0.09	ND	0.12 ± 0.21
1287	Acetoin	ND	0,21 ± 0.06	ND	0.49 ± 0.49	0.06 ± 0.05	0.61 ± 1.06	ND	0.15 ± 0.13
1304	Acetol	2.01 ± 1.87	0,23 ± 0.10	0.06 ± 0.01	0.10 ± 0.01	ND	0.05 ± 0.08	ND	0.19 ± 0.05
1339	6-Methyl-5-hepten-2-one	0.17 ± 0.01	0,72 ± 0.60	0.14 ± 0.01	0.65 ± 0.39	0.24 ± 0.03	0.27 ± 0.05	0.14 ± 0.01	0.40 ± 0.19
1390	Methyl heptyl ketone	ND	0.38 ± 0.05	ND	0.45 ± 0.18	0.15 ± 0.05	0.04 ± 0.08	ND	0.30 ± 0.08
1396	Nonanal	2.37 ± 1.09	1.21 ± 0.40	0.95 ± 0.19	1.92 ± 1.43	3.54 ± 0.69	3.10 ± 1.23	0.60 ± 0.02	9.22 ± 6.93
1467	Furfural	5.95 ± 1.71	11.32 ± 2.49	6.25 ± 0.46	3.61 ± 2.40	7.40 ± 0.46	2.27 ± 2.41	ND	8.71 ± 6.64
1501	Decanal	1.07 ± 0.41	0.70 ± 0.31	0.45 ± 0.07	0.68 ± 0.11	1.46 ± 0.13	1.00 ± 0.30	0.38 ± 0.09	1.93 ± 0.64
1510	1,4-Dimethyl-δ-3-tetrahydroacetophenone	ND	0.22 ± 0.13	ND	ND	ND	0.63 ± 1.09	0.28 ± 0.01	ND
1535	Benzaldehyde	4.79 ± 0.48	4.31 ± 1.91	1.50 ± 0.18	3.62 ± 0.87	3.49 ± 0.66	2.55 ± 1.56	1.16 ± 0.01	1.22 ± 0.54
1547	Lilac aldehyde A	ND	0.40 ± 0.02	ND	0.23 ± 0.03	0.65 ± 0.57	0.33 ± 0.58	ND	0.52 ± 0.89
1579	5-Methil-2-furaldehyde	0.82 ± 0.39	1.02 ± 0.27	ND	0.49 ± 0.15	0.44 ± 0.10	0.10 ± 0.18	ND	0.51 ± 0.34
1670	Acetophenone	1.05 ± 0.03	2.26 ± 0.74	ND	ND	ND	1.23 ± 1.42	1.04 ± 0.14	ND
1738	Piperitone	1.40 ± 0.02	1.57 ± 0.19	ND	ND	ND	ND	ND	ND
1747	Carvone	ND	ND	ND	0.72 ± 0.56	0.21 ± 0.21	ND	ND	0.58 ± 0.62
1795	Cuminaldehyde	0.09 ± 0.01	0.16 ± 0.11	0.39 ± 0.02	0.08 ± 0.13	0.08 ± 0.13	0.29 ± 0.30	ND	0.71 ± 0.66
1830	β-Damascenone	0.55 ± 0.05	0.47 ± 0.12	0.21 ± 0.04	ND	ND	0.19 ± 0.17	ND	ND
1855	Geranyl acetone	0.07 ± 0.03	0.10 ± 0.02	ND	ND	0.13 ± 0.04	0.06 ± 0.05	0.35 ± 0.01	0.19 ± 0.14
1990	2,5-Furandicarboxaldehyde	ND	ND	ND	0.11 ± 0.19	0.29 ± 0.18	0.07 ± 0.13	ND	ND
Alcohols									
1090	Isobutyl alcohol	ND	0.44 ± 0.33	ND	0.04 ± 0.06	1.50 ± 2.50	ND	ND	0.08 ± 0.14
1116	2-Pentanol	ND	ND	ND	ND	0.25 ± 0.30	ND	ND	0.23 ± 0.29
1138	1-butanol	ND	0.55 ± 0.02	ND	0.07 ± 0.12	ND	ND	ND	0.13 ± 0.11
1196	3-hexanol	ND	ND	ND	ND	0.09 ± 0.10	ND	ND	ND
1201	3-Methyl-1-butanol	ND	3.31 ± 1.11	ND	7.30 ± 6.41	1.50 ± 0.94	ND	ND	1.33 ± 1.25
1244	1-Pentanol	ND	ND	1.01 ± 0.07	ND	ND	ND	ND	ND

Table 3 (continued)

Esters					Honey sample				
RI	Name	Rhododendron	Chestnut	Lavandula	Astragalus	Chaste tree	Polyfloral	Oak	Pine
1251	3-Methyl-3-buten-1-ol	ND	1.02 ± 0.54	ND	0.82 ± 0.28	0.60 ± 0.14	0.46 ± 0.80	0.85 ± 0.07	1.35 ± 0.42
1290	Octanol	0.41 ± 0.01	0.43 ± 0.11	0.61 ± 0.07	0.42 ± 0.14	0.37 ± 0.24	0.74 ± 0.27	ND	1.10 ± 0.78
1313	2-Heptanol	0.23 ± 0.16	0.45 ± 0.10	0.46 ± 0.06	0.05 ± 0.04	0.05 ± 0.09	0.18 ± 0.04	ND	0.16 ± 0.28
1245	n-Hexanol	0.17 ± 0.02	0.61 ± 0.36	17.52 ± 1.94	0.44 ± 0.28	0.22 ± 0.07	0.29 ± 0.14	0.60 ± 0.01	0.51 ± 0.20
1278	cis-3-Hexene-1-ol	0.14 ± 0.01	1.08 ± 0.61	0.05 ± 0.01	0.14 ± 0.12	0.29 ± 0.33	0.34 ± 0.16	0.82 ± 0.05	0.12 ± 0.10
1385	n-Octan-3-ol	ND	0.22 ± 0.02	ND	0.32 ± 0.08	0.04 ± 0.04	0.13 ± 0.23	ND	0.13 ± 0.02
1411	2-Octanol	ND	ND	ND	ND	ND	ND	ND	0.12 ± 0.03
1459	2,6-Dimethyl-7-octen-2-ol	0.10 ± 0.01	0.11 ± 0.04	0.07 ± 0.01	0.28 ± 0.14	0.27 ± 0.08	4.18 ± 6.78	0.21 ± 0.02	0.29 ± 0.11
1482	2-Ethylhexanol	2.11 ± 0.89	1.15 ± 0.15	0.62 ± 0.03	1.06 ± 0.15	1.28 ± 0.23	1.21 ± 0.37	1.72 ± 0.05	1.53 ± 0.53
1510	6-Methyl-5-hepten-2-ol	0.78 ± 0.02	1.02 ± 0.05	ND	1.03 ± 0.08	0.91 ± 0.06	0.90 ± 0.23	0.66 ± 0.01	1.76 ± 0.38
1553	1-Octanol	ND	0.88 ± 0.36	ND	0.65 ± 0.23	0.46 ± 0.07	0.62 ± 1.08	ND	1.61 ± 0.64
1584	D-Fenchyl alcohol	ND	ND	ND	0.10 ± 0.04	0.03 ± 0.06	ND	ND	0.11 ± 0.01
1605	n-Nonyl alcohol	ND	1.40 ± 1.68	0.32 ± 0.04	5.19 ± 0.73	35.46 ± 6.10	5.10 ± 7.96	ND	7.78 ± 3.96
1688	1,8-menthadien-4-ol	0.04 ± 0.02	0.16 ± 0.02	ND	ND	0.03 ± 0.02	0.43 ± 0.35	0.51 ± 0.01	0.13 ± 0.15
1715	Decyl alcohol	0.08 ± 0.01	0.20 ± 0.01	ND	0.02 ± 0.04	0.07 ± 0.02	0.15 ± 0.09	0.55 ± 0.09	0.30 ± 0.16
1810	2-Hexadecanol	ND	2.98 ± 0.06	ND	0.02 ± 0.03	0.03 ± 0.05	0.04 ± 0.08	ND	0.13 ± 0.12
1845	p-Cymen-8-ol	ND	0.58 ± 0.08	0.06 ± 0.02	ND	0.06 ± 0.01	0.06 ± 0.10	1.42 ± 0.05	ND
1860	2-Methoxy-phenol	ND	ND	ND	0.07 ± 0.01	0.30 ± 0.17	0.05 ± 0.09	ND	0.23 ± 0.23
1876	Benzyl alcohol	0.60 ± 0.19	1.11 ± 0.10	0.04 ± 0.01	0.20 ± 0.08	0.11 ± 0.02	0.14 ± 0.08	0.86 ± 0.01	0.16 ± 0.04
1913	Phenethyl alcohol	0.76 ± 0.25	0.68 ± 0.29	0.18 ± 0.03	2.23 ± 1.05	0.54 ± 0.12	0.60 ± 0.54	1.00 ± 0.02	0.26 ± 0.15
1925	2,6-ditert-butyl-3-methyl-phenol	ND	ND	ND	0.03 ± 0.05	0.21 ± 0.19	0.29 ± 0.51	ND	1.91 ± 3.31
1985	n-Dodecanol	0.14 ± 0.02	0.31 ± 0.20	ND	0.09 ± 0.01	0.34 ± 0.08	0.16 ± 0.27	ND	0.44 ± 0.35
2015	Phenol	0.12 ± 0.07	0.15 ± 0.03	ND	0.02 ± 0.03	0.09 ± 0.05	0.09 ± 0.08	ND	0.06 ± 0.06
2045	3-Phenylpropanol	0.36 ± 0.08	0.11 ± 0.02	ND	ND	ND	ND	ND	ND
2225	2,4-Di-tert-butylphenol	ND	0.11 ± 0.01	ND	0.14 ± 0.12	0.13 ± 0.10	0.05 ± 0.09	ND	0.05 ± 0.09
Terpenes									
1120	Verbenene	0.24 ± 0.01	0.50 ± 0.02	0.28 ± 0.03	ND	ND	0.28 ± 0.24	0.09 ± 0.16	0.19 ± 0.09
1021	α-Pinene	ND	ND	ND	0.36 ± 0.63	0.77 ± 0.34	ND	ND	1.18 ± 0.21
1138	o-Xylene	0.12 ± 0.01	0.89 ± 0.84	0.19 ± 0.02	0.15 ± 0.07	0.16 ± 0.20	0.25 ± 0.23	0.06 ± 0.10	0.15 ± 0.05
1197	dl-Limonene	0.20 ± 0.02	0.28 ± 0.12	0.48 ± 0.03	0.16 ± 0.27	0.42 ± 0.13	0.64 ± 0.75	0.76 ± 1.31	ND
1255	Styrene	0.27 ± 0.09	0.29 ± 0.27	ND	ND	0.13 ± 0.02	ND	ND	ND
1265	Cymol	ND	ND	ND	ND	0.18 ± 0.06	0.08 ± 0.14	ND	0.07 ± 0.12
1276	p-Cymene	0.08 ± 0.08	0.49 ± 0.02	0.45 ± 0.05	0.09 ± 0.15	0.01 ± 0.02	0.41 ± 0.45	0.37 ± 0.64	0.13 ± 0.09
1287	α-Terpinolene	ND	0.11 ± 0.01	0.14 ± 0.01	ND	ND	0.06 ± 0.10	0.14 ± 0.25	ND
1471	trans-Linalool oxide	5.52 ± 1.44	5.12 ± 0.39	7.95 ± 6.01	ND	ND	3.52 ± 3.26	2.80 ± 4.85	ND
1488	β-Ionone	0.24 ± 0.02	0.41 ± 0.11	ND	0.30 ± 0.07	0.43 ± 0.09	0.22 ± 0.23	0.07 ± 0.12	0.41 ± 0.47
1445	Linalool	0.99 ± 0.44	1.31 ± 0.69	1.25 ± 0.20	1.43 ± 0.63	0.98 ± 0.11	1.18 ± 0.37	0.41 ± 0.70	0.58 ± 0.60
1603	Hotrienol	7.93 ± 5.64	6.23 ± 1.16	8.87 ± 0.91	2.05 ± 0.23	0.01 ± 0.02	6.56 ± 6.52	1.39 ± 2.41	1.14 ± 0.83
1697	α-terpineol	ND	0.38 ± 0.05	ND	0.51 ± 0.41	0.26 ± 0.23	0.16 ± 0.28	ND	0.46 ± 0.24
1701	4-Ketoisophorone	ND	1.06 ± 0.01	ND	0.50 ± 0.32	0.25 ± 0.22	0.25 ± 0.44	ND	0.79 ± 0.84
1738	Phellandral	0.08 ± 0.02	0.27 ± 0.02	1.46 ± 0.20	ND	0.08 ± 0.02	0.13 ± 0.23	ND	0.04 ± 0.06
1948	β-ionone	ND	ND	ND	0.02 ± 0.04	0.08 ± 0.01	ND	ND	ND
2153	Carvacrol	ND	0.09 ± 0.01	ND	0.04 ± 0.08	ND	ND	0.11 ± 0.20	0.04 ± 0.06
2193	o-Aminoacetophenone	0.14 ± 0.01	0.82 ± 0.03	ND	ND	0.02 ± 0.04	0.04 ± 0.06	ND	0.04 ± 0.06
Acids									
1443	Acetic acid	ND	8.52 ± 0.18	ND	6.15 ± 0.42	0.39 ± 0.37	3.27 ± 5.66	ND	3.06 ± 1.95
1635	Isobutyric acid	ND	0.78 ± 0.48	ND	0.10 ± 0.17	0.10 ± 0.09	0.27 ± 0.47	ND	0.28 ± 0.34
1662	2-Methylbutanoic acid	ND	4.58 ± 0.18	ND	1.60 ± 1.03	ND	1.39 ± 2.41	ND	1.11 ± 0.52
1636	n-Hexanoic acid	0.42 ± 0.03	0.63 ± 0.35	0.70 ± 0.02	0.54 ± 0.31	0.50 ± 0.05	0.86 ± 0.39	0.14 ± 0.24	0.73 ± 0.39
1920	2-Ethyl-hexanoic acid	0.15 ± 0.01	0.19 ± 0.03	0.24 ± 0.05	0.43 ± 0.21	0.51 ± 0.11	0.15 ± 0.06	0.04 ± 0.08	0.19 ± 0.16
1944	Heptanoic acid	0.12 ± 0.04	0.43 ± 0.36	0.27 ± 0.04	0.07 ± 0.12	0.18 ± 0.07	0.14 ± 0.06	0.06 ± 0.10	0.18 ± 0.08
2045	Octanoic acid	0.91 ± 0.03	1.00 ± 0.28	0.42 ± 0.04	1.25 ± 0.88	0.81 ± 0.15	0.92 ± 0.35	0.33 ± 0.57	2.19 ± 0.68
2136	Nonanoic acid	0.50 ± 0.21	1.53 ± 0.48	0.37 ± 0.02	1.10 ± 0.84	1.16 ± 0.15	0.84 ± 0.20	ND	2.39 ± 0.26

Table 3 (continued)

Esters					Honey sample				
RI	Name	Rhododendron	Chestnut	Lavandula	Astragalus	Chaste tree	Polyfloral	Oak	Pine
2218	Decanoic acid	0.11 ± 0.06	0.24 ± 0.12	0.11 ± 0.01	0.47 ± 0.32	0.19 ± 0.09	0.31 ± 0.12	0.09 ± 0.16	0.43 ± 0.17

Data represent the mean values and standard deviations from duplicate injections. *RI* Retention indice, *ND* not detected

has been described as one of the most representative compounds for distinguishing between different flower origins [3].

Terpenes are known as the major group of secondary metabolites in a great variety of aromatic plants. Bees collect nectar from aromatic plants to produce honey, and terpenes are found in the resulting honeys. Regular monoterpenes derived from geranyl pyrophosphate, such as linalool derivatives, including linalool, trans-linalool oxides and hotrienol, are the most common honey terpenes [34]. Among the terpenes, α-pinene was identified in all pine and chaste tree honey samples in the present study. Silici [35] reported that the presence of some compounds (e.g., α-pinene) can be considered an indicator of unifloral honey, and this compound was one of the main components of the volatile profile of pine honeys. More recent study revealed that α-pinene can be evaluated as one of the important volatiles for a rarely studied honeydew honey, Greek *Quercus ilex* honey [36]. Some linalool derivatives, such as linalool oxide, can be produced during honey storage [6]. This compound is one of the important terpene compounds commonly found in lavender, coriander, rosemary, and citrus honeys [34]. Trans-linalool oxide was found at a higher rate than other terpene compounds, especially in lavandula. Another terpenic compound is hotrienol, which bestows a sweet flower aroma reminiscent of the plant source. Hotrienol was identified among the terpenic compounds present at significantly high concentrations in lavandula, rhododendron, and chestnut honeys. Researchers have suggested that this compound may contribute to the differentiation of lavender honey [5]. Hotrienol is also known as a compound derived from thermal degradation of honey, although there are also studies reporting its occurrence without prolonged storage or heat treatment [37].

Five volatile acid compounds were identified in all the samples analyzed, albeit at different concentrations—n-hexanoic acid, octanoic acid, 2 ethyl-hexanoic acid, heptanoic acid, and decanoic acid. Acetic acid was found at significant levels in chestnut, astragalus, polyfloral, and pine honeys. Short chain acids, such as acetic acid, bestow spicy flavors and aromas, while butanoic acid and hexanoic acids are linked to a rancid aroma [38]. Acetic acid has also been identified for chaste tree honey, although in lesser amounts. Also, in another study, this compound was found in pine and thyme honeys from Greece [39]. Jánošková et al. [40] identified acetic acid as one of the markers for Slovak honeydew honey. This compound has previously reported as one of the main volatile components of acacia, buckwheat, chestnut, eucalyptus, fir tree, honeydew, lavender, lime tree, orange, rape, rhododendron, rosemary, sunflower and thyme honeys [41].

Data clustering with PCA

The first two principal components (PC) covered 98.10% of total variance (PC1 = 93.77%, PC2 = 4.33%). The most important first PC1 was strongly associated with ethyl butanoate, 3-methoxy-1-butyl acetate, ethyl benzeneacetate, 3-methyl-1-butanal, α-pinene, diastase, optical rotation, 2,3-butanedione, and n-nonyl alcohol. The second component (PC2) was strongly defined by n-hexanal, 1-butanol, piperitone, conductivity, p-cymen-8-ol, and ethyl decanoate. Figure 1 shows the PCA results of the data set projected onto the first two PCs. Oak, pine, chestnut, rhododendron, astragalus, and lavandula were correctly differentiated. No overlaps occurred among the honey types. Honeydew honeys were found in the right quadrant, whereas pure blossom honeys such as chestnut, rhododendron, astragalus, and lavandula were located on the left. Polyfloral honeys, the only sample group from several plant species, occupied positions in both the bottom right and bottom left quadrants. This can be explained by the dissimilar composition of the studied polyfloral honeys, since they contain highly variable chemical compounds.

An additional figure was created using rotated component matrix data (Fig. 1b). This shows compound loadings of the PCA analysis for PC1 and PC2. Although important compound loadings are visualized to assist with honey differentiation, not all of the compound loadings are visualized. This is because principal component analysis has been carried out with large data sets (i.e., biochemical characteristics and volatile compounds represent significant differences). The position of variables in the divided parts of the plot are worthy of note. The bottom part of the loading plot shows that *n*-hexanol, heptanal, 2,6-dimethyl-7-octen-2-ol, *α*-pinene, and 2-methoxy-phenol are responsible for differentiation between the oak and pine honeys. While pine honey contained *n*-heptanal, *α*-pinene and 2-methoxy-phenol, these compounds were not present in oak honey. The PCA results, therefore, suggest that those compounds might be used to discriminate between oak and pine honeys. The loading plot also shows that certain compounds, such as ethyl acetate

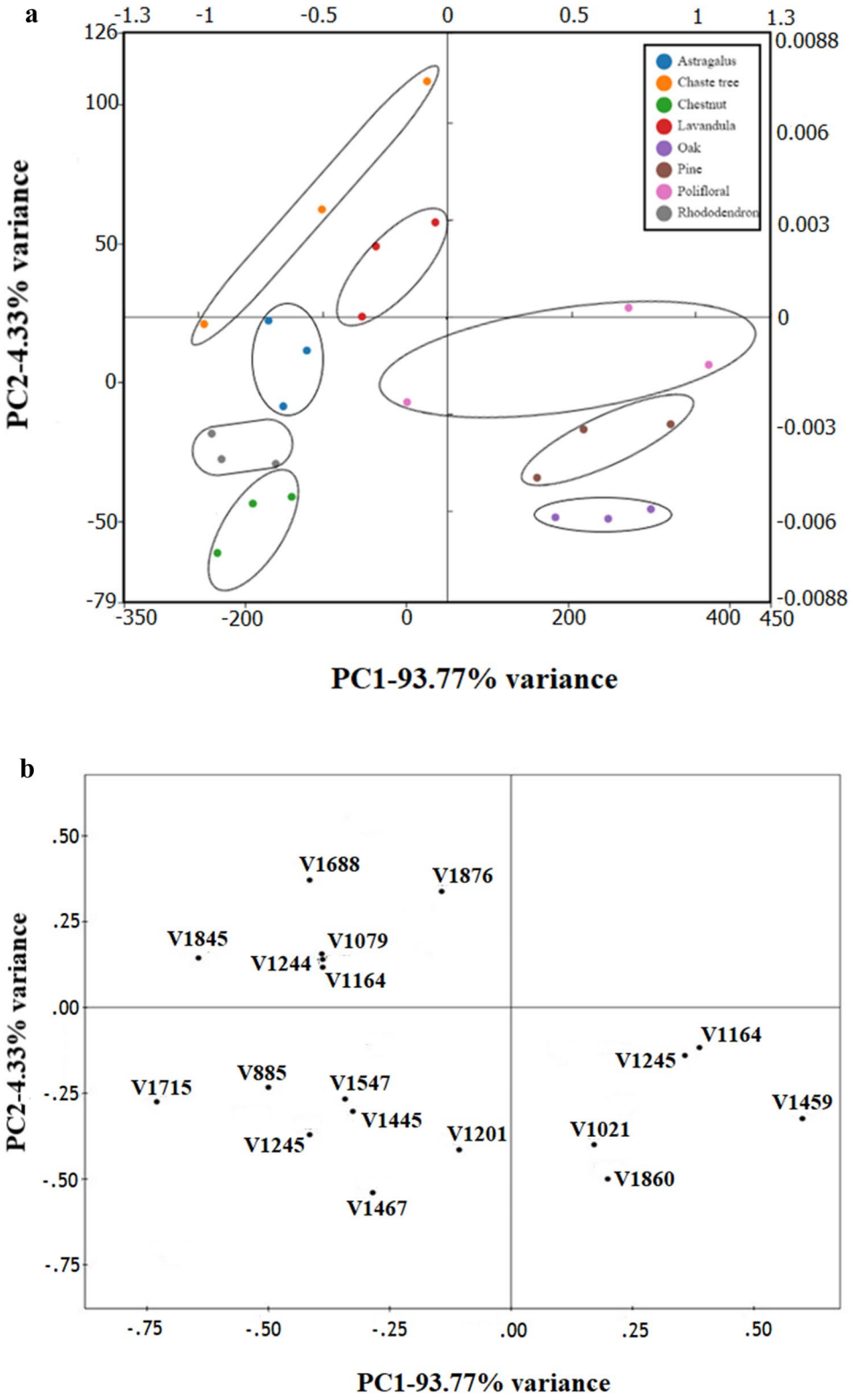


Fig. 1 **a** PCA plot of honey samples. **b** Volatile compound loadings of the PCA analysis for PC1 and PC2. Compounds are shown with RI (retention index)

and linalool, are responsible for differentiation of honeys located on the bottom left quadrant. 3-Methyl-1-butanol and lilac aldehyde A are some of the components that contribute to the grouping of rhododendron and chestnut honeys. The upper-left side is noteworthy for the presence of three compounds including 1-pentanol, n-hexanal and *n*-heptanal. 1-pentanol was only identified in lavandula honey. Additionally, *n*-hexanal and *n*-heptanal contents in lavandula were

considerably higher than in the other honeys. Proximity between loading plots indicates that conspicuous *n*-hexanal and *n*-heptanal values may be capable of use for can differentiating lavender honey.

Conclusion

Honey is often the target of adulteration due to its high commercial value and nutritional benefits. This widespread and ongoing adulteration has made authentication and classification highly popular fields for researchers. Monofloral honeys of confirmed botanical origins are today regarded as the highest quality product, and the marketing of these is of great importance for consumers. However, honey authentication is also challenging because of its complex nature. Depending on botanical origins, the volatile composition and biochemical characteristics of honeys vary. Volatile aroma compounds, consisting of 15 esters, 30 aldehydes and ketones, 31 alcohols, 18 terpenes, and nine acids, were identified from eight types of different Turkish honey samples. The PCA results showed that the procedure is a promising one by explaining 98.10% of the total variance for discriminating Turkish honeys from different floral sources, based on volatile composition and physicochemical data.

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Author contributions Oktay YILDIZ: Conceptualization, Methodology, Software. Sevgi KOLAYLI: Data curation, Writing—Original draft preparation. Atiye DEGIRMENCI: Visualization, Investigation. A. Adnan HAYALOGLU: Supervision. Hacer GURKAN, Mehtap ER KEMAL: Software, Validation. Didem SAHINGIL: Writing—Reviewing and Editing.

Declarations

Conflict of interest The authors declare that they have no known competing fnancial interests or personal relationships that could have appeared to infuence the work reported in this manuscript.

Compliancewith ethics requirements The research did not involve human participants and/or animals. Ethical approval was not required for this research.

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