Food Chemistry 180 (2015) 133–141

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles

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ARTICLE INFO

Article history:
Received 1 November 2014
Received in revised form 2 February 2015
Accepted 5 February 2015
Available online 14 February 2015

Keywords:
Honey
Unifloral
Honeydew
Phenolics
Antioxidant
Turkey
Apitherapy

ABSTRACT

This study investigated some physico-chemical and biochemical characteristics of different honey types belonging to Turkish flora. Sixty-two honey samples were examined on the basis of pollen analyses, including 11 unifloral honeys (chestnut, heather, chaste tree, rhododendron, common eryngo, lavender, Jerusalem tea, astragalus, clover and acacia), two different honeydew honeys (lime and oak), and 7 different multifloral honeys. Electrical conductivity, moisture, Hunter color values, HMF, proline, diastase number, and sugar analyses of the honey samples were assessed for chemical characterization. Some phenolic components were analyzed by reverse phase high performance liquid chromatography (RP-HPLC) to determine honeys' phenolic profiles. Total phenolic compounds, total flavonoids, ferric reducing antioxidant capacity (FRAP) and 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity were measured as antioxidant determinants. The study results confirm that physico-chemical and biological characteristics of honeys are closely related to their floral sources, and that dark-colored honeys such as oak, chestnut and heather, have a high therapeutic potential.

1. Introduction

The adverse effects on human health of synthetic drugs and chemicals in the age of technology have encouraged the use of more traditional and natural methods. Therapeutic techniques using bee products that protect and strengthen the immune system are known as apitherapy. Honey, a rediscovered natural product, has also begun being used for numerous purposes.

Although the great majority of the dry weight of honey (95–98%) consists of carbohydrates, 2–5% is made up of various secondary metabolite agents and minerals. The main sugars in the structure are fructose and glucose, although it also contains small quantities of mono-, di- and trisaccharides and oligosaccharides. Some of these are criteria for honey purity recognized in international food codices (IHC, CEU, TSE etc.) (Codex, Standard 12-1981), but these do not indicate honey's bioactive potential and apitherapic functions. The true quality of honey is associated with the presence, variety and amounts of its bioactive compounds, and this again depends on the geographic and floral structure of the region in which it is produced. Studies show that the great majority of the bioactive compounds in honey consist of molecules with phenolic structures, such as phenolic acids, flavonoids, procyanidins and anthocyanins (Küçük et al., 2007; Sahin, Aliyazıcıoğlu, Yıldız, Kolayli, & Supuran, 2011; Tezcan, Kolayli, Sahin, Ulusoy, & Erim, 2011).

Both animal studies and clinical trials in different parts of the world are providing highly promising results regarding the healing potential of honey (Kassım et al., 2012; Yıldız et al., 2013). For example, with its high antimicrobial activity, New Zealand Manuka honey is used in the treatment of wounds and burns. Manuka is a honey classified on the basis of the amount of methyl syringate it contains and is known for its dark color and high phenolic content (Jonathan et al., 2010). Similar to Manuka honey, other honeys such as Tualang and Gelam are also known to possess high levels of biological activity and to have potential for use in apitherapy (Kassım et al., 2012; Küçük et al., 2007). Many studies have suggested that the antioxidant and antimicrobial activities of honey samples correlate with the total phenolic contents and the color pigments within samples (Kassım et al., 2012; Tezcan et al.,

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http://dx.doi.org/10.1016/j.foodchem.2015.02.024
0308-8146/

2011). Over the last two decades, research into the role of apitherapic products in the prevention and treatment of human diseases has intensified, and their antioxidant, antibacterial, antitumoral, and anti-inflammatory potentials have been revealed (Nasuti, Gabbianelli, Falcioni, & Cantalamessa, 2006).

Due to features such as its geographical position, climatic conditions and three seasons of the year being suited to honey production, Turkey is one of the richest regions of the world in terms of honey production and variety. It is home to a wide variety of nectar and honeydew honey types, both unifloral and multifloral. The purpose of this study was to reveal the physical and chemical characterizations, and antioxidant and antimicrobial activities of honeys from different flora produced in Turkey and to identify honeys with a high apitherapy potential for future studies.

2. Materials and methods

2.1. Chemicals

2,4,6-Tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu's phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 5-hydroxymethlfurfural (HMF) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

All chemical standards were HPLC-grade pure, and the common phenolic compounds and sugars were obtained from Sigma–Aldrich (Munich, Germany). Of the HPLC grade organic reagents, acetonitrile was supplied by Sigma–Aldrich Co. (St. Louis, MO, USA) and methanol by Merck KGaA, (Darmstandt, Germany). HPLC syringe filters (RC-membrane, 0.45 μm) were purchased from Sartorius Minisart RC 15, Sartorius (Germany).

2.2. Honey samples

Thirteen varieties of unifloral honey, chestnut (*Castanea sativa* Mill.), heather (*Calluna vulgaris* L.), chaste tree (*Vitex agnus-castus* L.), rhododendron (*Rhododendron ponticum* L.), lime (*Tilia platyphyllos*), clover (*Trifolium* spp. L.), oak (*Quercus robur* L.), pine (*Pinus brutia* L.), lavender (*Lavandula stoechas* L.), Jerusalem tea (*Phlomis armeniaca* Willd.), astragalus (*Astragalus microcephalus*), black locust (*Robinia pseudoacacia*) and common eryngo (*Eryngium campestre* L.), and seven different multifloral honeys from Turkey obtained by experienced beekeepers in the 2011–2012 harvest season were included in the study.

2.3. Melissoplaynological analysis of honey samples

Honey samples were classified on the basis of melissopalynological characterization according to their specific botanical variety. The preparation of honey samples followed the standardized method described by Louveaux, Maurizio, and Vorwohl (1978).

The pollen types present in the honey samples were identified, counted, and classified, according to their percentages, as dominant pollen (45% or more of the total pollen grains counted) and, secondary pollen (16–44%), important minor pollen (3–15%), and minor pollen (less than 3%). Counts were expressed as percentages after counting a minimum 1000 pollen grains on four slides from sample.

2.4. Analytical examinations of honey samples

Some physicochemical characteristics of the honeys were in agreement with European Union (Bogdanov et al., 1997). The color value of the honeys was determined using a Hunter spectrometer (CR-400, Minolta, Osaka, Japan). Moisture was measured using a refractometer (Atago, Tokyo, Japan), electrical conductivities with a conductometer (WTW inoLab Cond/720, Germany), and optical activity or rotation with a polarimeter (Beta PPP7, England).

Sugar analysis of samples was performed using a refractive detector (RID) with HPLC (Elite LaChrom, Hitachi, Japan) and a reverse phase–amide column (200/4.6 Nucleosil 100-5 NH_2). Quantitative and qualitative sugar analyses were performed using the method described by Ozturk, Tuncel, and Tuncel (2007). The calibration curves of all analyzed sugars were between 0.994 and 1.000.

Hydroxymethylfurfural (HMF) was measured with HPLC-UV (Elite Lachrom Hitachi, Japan) using a C_{18} column of LiChroCART® 250-4 RP (10 μm) (Jeuring & Kupper, 1980). Proline content was measured using spectrometric assay (Ough, 1960). Diastase activity was determined with the spectrophotometric method using a buffered solution of soluble starch and honey incubated in a thermostatic bath at 40 °C (Bogdanov et al., 1997).

2.5. Honey extraction for antioxidant activity and phenolics analysis

Methanolic extracts of the honeys were used for antioxidant analyses. Approximately 15 g honey was placed in a falcon tube (50 mL) and 50 mL 99% methanol was added. The mixture was continuously stirred with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h. Particles were removed with filter paper. The final volume of the solution was adjusted with methanol. The methanolic extract was divided into two parts, the first being used for antioxidant tests and the second for phenolic analysis. Liquid–liquid extraction procedure was applied to this second part for phenolic determining (Akyuz, Sahin, Islamoglu, Kolayli, & Sandra, 2014).

2.6. Analysis of phenolic compounds by HPLC

Eighteen standards of phenolic compounds were analyzed using HPLC (Thermo Finnigan Surveyor), in a UV–Vis detector supplying a double wavelength simultaneously. Phenolic profile was determined according to Akyuz et al. (2014). For quantitative determining, each phenolic component calibration curves were between 0.998 and 1.000.

2.7. Determination of total phenolic content (TPC) and total flavonoids

TPCs of the methanolic extracts were determined following the Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). Folin assay was also based on all phenolic contents including phenolic acids, flavonoids, and anthocyanins in the aquatic solution. This gives a blue-color complex whose maximum absorbance can be read at 760 nm.

The amount of total flavonoid was measured with a spectrophotometric method at 415 nm as reported previously (Fukumoto & Mazza, 2000) using quercetin as standard.

2.8. Determination of total antioxidant capacity and free radial scavenging activity

The reducing ability of ferric tripyridyltriazine (Fe-III-TPTZ) complex was used for total antioxidant capacity assay (Benzie & Strain, 1996). And also, trolox was used as positive control to construct a reference curve (62.5–1000 μM).

The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was used to determine the radical scavenging activity of the methanolic honey samples. The colorimetric test was assayed using the Molyneux method (2004). DPPH radical has a purple color which decays in the presence of antioxidant agents, thus the change of the absorbance is monitored at 517 nm.

2.9. Statistical analysis

Data were tested using SPSS (version 9.0 for Windows 98, SPSS Inc.). Regression and correlation analysis were performed with Kruskal–Wallis and Pearson correlation analysis as a non-parametric test. Significance was set at $p < 0.05$.

3. Results and discussion

3.1. Physicochemical parameters

The common names, floral sources, regional names and regions of collection of honey samples collected from various regions of Turkey and subjected to melissopalynological analysis are summarized in Table 1. Excluding the 7 multifloral types of honey, 11 honeys, chestnut, astragalus, heather, clover, lavender, lime, Jerusalem tea, common eryngo, chaste tree, rhododendron, and acacia, were tagged as monofloral by determining the frequency classes of pollen type using microscopic analysis only. In addition, the botanical origins of pine and oak honeys were determined using palynological tests, together with the honey's optic rotation values and electrical conductivities. In contrast to floral honeys, honeydew honeys exhibit positive rotation values, and the honeys used in this study also had positive rotation values. Identification of honeydew honey type was based on beekeepers' declarations. Seven different multifloral honeys were used in the study, and their dominant pollen families are also shown in Table 1.

The physicochemical analysis results for the honeys studied are shown as arithmetical mean plus standard deviation in Table 2. Honeys of different floral origins possessed different color parameters. The Tritium color values of the honeys were expressed as L^* for darkness/lightness (0 black, 100 white), a^* ($-a$ greenness, $+a$ redness), and b^* ($-b$ blueness, $+b$ yellowness) (Anupama, Bhat, & Sapna, 2003). The L values of the samples ranged from 40 to 88, with lower L value indicating a darker honey color. Oak, heather and chestnut honeys exhibited the lowest L values and were defined as dark-colored. Acacia was the lightest-colored honey, and forest rose, multifloral honeys, lavender, lime, Jerusalem tea, and astragalus were also included in the light-colored honey class. The highest greenness value ($-a$ value) was determined in acacia honey and the highest redness in chestnut honey ($+a$ value). Pine and chestnut honeys exhibited the highest yellowness values (high b values) while clover, astragalus and Jerusalem tea were identified as those with the greatest blueness.

Honey color is the most important factor affecting its visual appearance, and is mostly dependent on nectar source and pollen content. While nectar source and pollen content determine honey color, various color pigments deriving from these (anthocyanins, phenolic acids, proanthocyanidins and flavonoids) and minerals constitute that basic color of honey (Gonzalez-Paramas, Garcia-Villanova, Gomez Barez, Sanchez Sanchez, & Ardanuy Albajar, 2007). All pure honeys gradually darken in color, due to various non-enzymatic caramelization reactions, known as Maillard reactions. HMF is one such reaction that affects honey darkening (Zalibera et al., 2008). Generally, dark-colored honeys have been reported to possess high levels of pigment, pollen, phenolic compounds, minerals and Maillard reaction products (Bertoncelj, Doberšek, Jamnik, & Golob, 2007; Tezcan et al., 2011). One study of Slovenian honeys showed that various forest honeys such as fir, forest and chestnut honeys were dark-colored (with low L values), while acacia was a light-colored honey (Bertoncelj et al., 2007). It has been suggested that oak, chestnut and heather are dark-colored honeys whose chromatic properties vary depending on amino acid composition, and that chromatic character is not a discriminating factor among these honeys (Gonzalez-Paramas, Garcia-Villanova, Gomez Barez, Sanchez Sanchez, & Ardanuy Albajar, 2007).

Honey's moisture levels varied between approximately 16–20%, and these were compatible with honey codices. The highest moisture levels were determined in heather and acacia honeys and the lowest in multifloral and honeydew honeys. Honey's electrical conductivities ranged between 0.30 and 1.50 mS/cm. Chestnut honey exhibited the highest conductivity, followed by honeydew honeys. Light-colored honeys such as acacia, lavender, clover, Jerusalem tea and astragalus exhibited lower conductivity. Electrical conductivity is a property of electron mobility and is largely correlated with honeys' mineral salt, organic acid and protein levels. Electrical conductivity is a significant discriminating parameter for honeydew honeys, which have greater conductivity than floral honeys (Alqarni, Owayss, Mahmoud, & Hannan, 2012). One study of honeys from various regions of Lithuania reported conductivity values between 0.34 and 0.89 mS/cm for floral honeys (Kaškoniene, Venskutonis, & Ceksteryt, 2010). Another study reported that the electrical conductivities of various mono- and heterofloral honeys ranged from 0.21 to 3.13 mS/cm and that dark-colored honeys exhibited higher conductivity (Alqarni et al., 2012). That same study reported a conductivity of 0.66 ms/cm for Manuka honey. These values are similar to those in our study. In contrast to floral honeys, chestnut honey has much higher electrical conductivity than the limit values in the honey codex. The idea that the high electrical conductivity of chestnut honey derives from its high mineral contents is supported by studies in the literature (Alissandrakis, Tarantilis, Pappas, Harizanis, & Polissiou, 2011). The high electrical conductivity of chestnut honey in contrast to floral honeys indicates that this may be an important marker for this honey.

Optical rotation was dextrorotatory in pine and oak honeys only, while blossom honeys exhibited levorotatory properties. In general, optical activity has been used to distinguish properties of honeydew honeys and adulterated honeys (Cavrar, Yıldız, Şahin, Karahalil, & Kolaylı, 2013).

3.2. Chemical parameters

Various recognized parameters were used for the evaluation of honey freshness (Bogdanov et al., 1997; Küçük et al., 2007; Mendes, Proença, Ferreira, & Ferreira, 1998). Diastase, one such parameter, plays a role in the digestion of starch and is secreted from the honeybee's stomach. In this study, honeys' diastase activities ranged from 6.30 to 13.20. Honeys with relatively low diastase activities in this study, such as heather, common eryngo, and clover honeys were older, and their activities gradually decreased. Another parameter of the freshness of honey is the amount of HMF contained. This, a non-enzymatic Maillard reaction product, is accepted at values below 0–40 mg/kg in honey codices. HMF values for all honeys in this study, apart from heather, were below 40 mg/kg. HMF values were elevated in some heather honeys because they were out of date, and a decrease in diastase activity was also observed in association with this. Mendes et al. (1998) reported that diastase activity and HMF levels of 25 brands honey by comparing expectation limits. Comparing our results with those from that study, the results may be considered consistent.

Honeys' proline values ranged between 282 and 845 mg/kg. The honeys with the highest proline values were heather, chestnut, chaste tree and astragalus, while acacia honey had the lowest proline content. Honey contains 20 amino acids, although proline is interestingly the amino acid present in the highest quantities. The amount of proline in honey is a marker of purity, and the level decreases significantly in adulterated honeys. The level of proline has been reported to vary according to the honey flora, but this is more closely associated with the bees' work performance

Table 1
Data from studies included in the identification markers.

Flora	Location	Dom. Pollen %	Predominant pollen	Local name	Sensory characteristics (colour, consistency)
Chestnut	Giresun/Eynesil	95	*Castanea sativa* Mill.	Kestane	Dark, liquid
Chestnut	Kastamonu	97	*Castanea sativa* Mill.	Kestane	Dark, liquid
Chestnut	Artvin	97	*Castanea sativa* Mill.	Kestane	Dark, liquid
Chestnut	Artvin/Borçka	98	*Castanea sativa* Mill.	Kestane	Dark, liquid
Chestnut	Trabzon/Araklı	97	*Castanea sativa* Mill.	Kestane	Dark, liquid
Chestnut	Trabzon/Of	92	*Castanea sativa* Mill.	Kestane	Dark, liquid
Chestnut	Zonguldak	94	*Castanea sativa* Mill.	Kestane	Dark, liquid
Astragalus	Çanakkale/Ecabat	51	*Astragalus microcephalus* Willd.	Geven	Pale amber
Astragalus	Çanakkale/Biga	54	*Astragalus microcephalus* Willd.	Geven	Yellow, liquid
Astragalus	Elazıg	55	*Astragalus microcephalus* Willd.	Geven	Yellow, liquid
Astragalus	Çanakkale	55	*Astragalus microcephalus* Willd.	Geven	Yellow, liquid
Astragalus	Diyarbakır	53	*Astragalus microcephalus* Willd.	Geven	Yellow, liquid
Heather	Çanakkale/Biga	98	*Calluna vulgaris*	Püren	Dark brown, liquid
Heather	Çanakkale	97	*Calluna vulgaris*	Püren	Dark brown, liquid
Heather	Çanakkale/Eceabat	80	*Calluna vulgaris*	Püren	Dark brown, liquid
Heather	Antalya	95	*Calluna vulgaris*	Püren	Dark brown, liquid
Heather	Muğla	97	*Calluna vulgaris*	Püren	Dark brown, liquid
Heather	Muğla	70	*Calluna vulgaris*	Püren	Dark brown, liquid
Clover	Çanakkale/Eceabat	45	*Trifolium* L.	Üçgül	Pale yellow, cream
Clover	Çanakkale	48	*Trifolium* L.	Üçgül	Pale yellow, cream
Clover	Çanakkale/Ayvacık	49	*Trifolium* L.	Üçgül	Pale yellow, cream
Lavender	Çanakkale/Lapseki	48	*Lavandula stoechas* L.	Lavanta, Karabaş	Pale amber, solid
Lavender	Çanakkale/Bayramiç	51	*Lavandula stoechas* L.	Lavanta, Karabaş	Pale amber, solid
Lavender	Çanakkale/Biga	50	*Lavandula stoechas* L.	Lavanta, Karabaş	Pale, crystallised
Lavender	Isparta	53	*Lavandula stoechas* L.	Lavanta, Karabaş	Pale, solid
Lavender	Antalya	46	*Lavandula stoechas* L.	Lavanta, Karabaş	Pale, solid
Lime	Kastamonu	47	*Tilia scop*	Ihlamur	Yellow, liquid
Lime	Sinop	45	*Tilia scop*	Ihlamur	Yellow, liquid
Lime	Samsun	45	*Tilia scop*	Ihlamur	Yellow, liquid
Jerusalem tea	Mersin	49	*Phlomis armeniaca* Willd.	Şalba	Pale amber, solid
Jerusalem tea	Adana	46	*Phlomis armeniaca* Willd.	Şalba	Pale amber solid
Jerusalem tea	Mersin	48	*Eryngium campestre* L.	Şalba	Pale amber solid
Common eryngo	Konya	45	*Eryngium campestre* L.	Tusi, dutsi	Amber, liquid
Common eryngo	Karaman	45	*Eryngium campestre* L.	Tusi, dutsi	Amber, liquid
Common eryngo	Hatay	45	*Vitex agnus castus*	Tusi,dutsi	Amber, liquid
Chaste tree	Karpuzlu	53	*Vitex agnus castus*	Hayıt	Amber, liquid
Chaste tree	Aydın/Çine	61	*Vitex agnus castus*	Hayıt	Amber, liquid
Chaste tree	Aydın	47	*Vitex agnus castus*	Hayıt	Amber, liquid
Chaste tree	Aydın	45	*Rhododendron ponticum*	Hayıt	Amber, liquid
Rhododendron	Bartın	58	*Rhododendron ponticum*	Ormangülü	Pale yellow, liquid
Rhododendron	Trabzon/Şalpazarı	55	*Rhododendron ponticum*	Ormangülü	Bright yellow, liquid
Rhododendron	Trabzon/Sis Dağı	67	*Rhododendron ponticum*	Ormangülü	Bright yellow, liquid
Rhododendron	Kastamonu	65	*Rhododendron ponticum*	Ormangülü	Bright yellow, liquid
Rhododendron	Trabzon	75	*Rhododendron ponticum*	Ormangülü	Bright yellow, liquid
Rhododendron	Rize	54	*Quercus robur* L.	Ormangülü	Bright yellow, liquid
Oak	Kırklareli/Demirköy	-	*Quercus robur* L.	Meşe	Turbid, Dark brown, liquid
Oak	Kırklareli/Kofçaz	-	*Quercus robur* L.	Meşe	Turbid, dark brown, liquid
Oak	Kırklareli/Gökyaka	-	*Pinus* L.	Meşe	Dark brown, liquid
Pine	Çanakkale/Gelibolu	-	*Pinus* L.	Çam	Dark brown, liquid
Pine	Muğla	-	*Pinus* L.	Çam	Brown, liquid
Pine	Muğla/Datça	-	*Pinus* L.	Çam	Brown, liquid
Pine	Çanakkale	-	*Robinia* L.	Çam	Brown, liquid

Table 1 (*continued*)

Flora	Location	Dom. Pollen %	Predominant pollen	Local name	Sensory characteristics (colour, consistency)
Acacia	Sinop	52	*Robinia* L.	Akasya	Yellow, liquid
Acacia	Ordu	45	*Robinia* L.	Akasya	White, solid
Acacia	Ordu/Fatsa	54	Fagaceae, fabaceae, asteraceae, rosacea	Akasya	Pale yellow, solid
Multifloral	Hatay	*	Fabaceae, lamiaceae, *A. microcephalus*	Yayla	Pale yellow, liquid
Multifloral	Kars/Digor	*	Fabaceae, trifolium, rubus	Yayla	Pale yellow, cream
Multifloral	Çanakkale/Lapseki	*	Lamiaceae, tiliaceae, rosaceae	Yayla	Amber, liquid
Multifloral	Eskişehir	*	Fagaceae, ericaceae, apiaceae tiliaceae, Phacelia, rosaceae, fabaceae	Yayla	Light amber, liquid
Multifloral	Trabzon/Akçaabat	*	Lamiaceae, asteraceae, phacelia,	Yayla	Amber, liquid
Multifloral	Gümüşhane/Şiran	*	Malvaceae, fabaceae, asteraceae, alliaceae	Yayla	Pale cream
Multifloral	Adıyaman	*	Phlomis armeniaca Willd.	Yayla	Pale yellow

*Dominant pollen was not present.

Table 2
Some physical and chemical parameters of different Turkish honeys.

Mean ± SD range samples	Hunter color parameters			Moisture %	Conductivity (mS/cm)	Optic rotation $[\alpha]_{20}$	Diastase (D.U)	HMF (mg/kg)	Proline (mg/kg)
	L	*a*	*b*						
Chestnut	47.59 ± 1.52^{b}	42.56 ± 5.88^{a}	81.52 ± 4.20^{a}	19.70 ± 1.33^{a}	1.50 ± 0.31^{a}	-2.73 ± 1.55^{b}	9.12 ± 2.99^{a}	9.28 ± 7.13^{a}	800 ± 178^{a}
Astragalus	74.80 ± 1.95^{e}	9.58 ± 0.62^{e}	5.11 ± 1.98^{c}	17.00 ± 0.76^{a}	0.48 ± 0.59^{c}	-1.99 ± 0.63^{b}	9.05 ± 1.37^{a}	4.60 ± 3.93^{a}	755 ± 130^{a}
Heather	46.05 ± 7.63^{b}	26.56 ± 1.16^{b}	68.00 ± 2.12^{b}	20.86 ± 2.13^{a}	0.80 ± 0.25^{b}	-2.63 ± 1.35^{b}	6.30 ± 4.13^{a}	62.24 ± 29.27^{b}	845 ± 42^{a}
Clover	70.35 ± 3.82^{e}	20.14 ± 1.55^{c}	5.89 ± 4.80^{c}	19.47 ± 2.44^{a}	0.32 ± 0.36^{c}	-2.57 ± 0.45^{b}	7.90 ± 1.30^{a}	10.92 ± 7.95^{a}	510 ± 64^{b}
Lavender	62.55 ± 2.73^{d}	15.20 ± 0.25^{b}	10.08 ± 2.78^{c}	17.15 ± 0.88^{a}	0.32 ± 0.12^{c}	-2.44 ± 1.10^{b}	8.44 ± 2.85^{a}	24.42 ± 6.78^{d}	615 ± 126^{b}
Lime	78.50 ± 4.20^{e}	3.10 ± 0.02^{g}	62.20 ± 2.45^{b}	19.80 ± 0.01^{a}	0.42 ± 0.26^{c}	-1.58 ± 0.23^{b}	9.20 ± 2.03^{a}	2.51 ± 0.24^{c}	514 ± 46^{b}
Jerusalem tea	73.66 ± 2.64^{e}	21.20 ± 2.30^{c}	6.92 ± 4.60^{c}	18.13 ± 0.42^{a}	0.55 ± 0.57^{c}	-3.06 ± 0.84^{b}	8.16 ± 2.90^{b}	8.48 ± 13.25^{a}	532 ± 138^{b}
Common eryngo	65.31 ± 2.58^{d}	14.23 ± 0.82^{b}	72.01 ± 2.68^{b}	17.95 ± 1.49^{a}	0.48 ± 0.17^{c}	-2.70 ± 0.52^{b}	7.80 ± 3.64^{a}	3.90 ± 2.36^{a}	455 ± 62^{c}
Chaste tree	49.79 ± 2.10^{b}	27.28 ± 0.20^{b}	69.00 ± 3.34^{b}	17.20 ± 0.40^{a}	0.26 ± 0.24^{c}	-2.26 ± 0.29^{b}	13.20 ± 1.38^{b}	12.17 ± 2.91^{a}	797 ± 129^{a}
Rhododendron	79.52 ± 1.21^{e}	7.50 ± 0.08^{f}	69.82 ± 5.16^{b}	19.50 ± 1.20^{a}	0.34 ± 0.12^{c}	-1.65 ± 0.55^{b}	10.65 ± 2.40^{a}	3.20 ± 1.10^{a}	746 ± 158^{a}
Oak	42.85 ± 1.26^{a}	34.59 ± 4.50^{a}	71.65 ± 4.10^{b}	17.07 ± 1.27^{a}	1.09 ± 0.16^{b}	0.74 ± 0.25^{a}	10.50 ± 2.22^{a}	0.61 ± 1.55^{c}	474 ± 115^{b}
Pine	54.38 ± 4.39^{c}	26.80 ± 2.63^{b}	89.45 ± 4.93^{a}	17.22 ± 0.96^{a}	0.99 ± 0.32^{b}	1.38 ± 1.40^{a}	11.60 ± 1.31^{b}	3.57 ± 2.06^{a}	437 ± 190^{b}
Acacia	88.50 ± 1.92^{f}	-1.55 ± 0.10^{h}	32.10 ± 2.24^{d}	20.80 ± 2.55^{a}	0.30 ± 0.25^{c}	-3.46 ± 0.64^{b}	12.60 ± 1.07^{a}	12.56 ± 5.21^{a}	282 ± 112^{b}
Multifloral	78.98 ± 4.50^{e}	13.50 ± 2.46^{b}	78.88 ± 6.56^{a}	16.54 ± 1.58^{a}	0.78 ± 0.62^{b}	-2.12 ± 0.33^{b}	9.07 ± 2.03^{a}	14.71 ± 12.10^{a}	482 ± 160^{b}

Different letters (a–h) in the same columns are significantly different at the 5% level ($p < 0.05$).

(Cotte et al., 2004). Experimental studies have reported that honeys from bees fed on sugar water exhibit low proline values (Cavrar et al., 2013). According to the honey codices, the desired level of proline in honey is 250 mg/kg, although the level is much higher in quality honeys, and the codices need to be revised (Codex, Standard 12-1981).

Analysis of honey sugar was performed with HPLC-RID and the % values for mono-, di- and trisaccharides per 100 g are shown in Table 3. The basic monosaccharides of honey are the reducing sugars fructose and glucose. F + G levels in this study ranged between 54.84 and 76.18, while the F/G ratio ranged between 1.16 and 2.44. Sucrose was detected in very few honeys, and the values determined were below 5%, compatible with the codices. Ribose, arabinose and galactose were not detected in any honeys. With the exception of chaste tree and clover, maltose was detected at levels of 1% or lower in all honeys. The disaccharides trehalose and melibiose were detected in some honeys, but not in others. Melezitose, a trisaccharide, was detected at levels of approximately 0.5% in all honeys apart from chaste tree and acacia, but at higher levels in oak and pine honey.

Honey's sugar composition depends on the floral and region origin (Andrade, Ferreres, & Amaral, 1997; Mendes et al., 1998). This is supported by our own research findings. Mean values for F/G ratio, trehalose, and melezitose in heather honeys were 1.98, 0.04, and 0.55, respectively, in our study. Mendes et al. (1998) investigated to the F/G ratio and other sugar components of some heather honeys and reported a range of F/G values of 1.03–1.33. Trehalose levels ranged from trace value to 21.7 g/kg, while no melezitose was detected.

3.3. Phenolic contents

In this study, honeys' phenolic compounds were determined as TPC, total flavonoids and phenolic profile. TPC is at the same time a marker of honey's antioxidant capacity and is generally used as an antioxidant test. Honey's total phenolic contents and other antioxidant activity tests are shown in Table 4. Honey's TPC levels varied widely, between 16.02 and 120.04 mg GAE/100 g. Moreover, statistical data show that honeys divide into four separate categories. The first category consists of oak, heather and chestnut honey and contains the highest level of phenolic contents. The fourth category consists of acacia and multifloral honeys alone and has the lowest phenolic contents. The great majority of honeys are in the second category, with TPC ranging between 30 and 60 mg GAE/100 g. Honeys in the first category also had the darkest color, while those in the fourth category were lightest in color.

Blossom honeys are produced from the nectars of flower-bearing plants, while honeydew honeys are more the product of the digestive by-products from aphids collected by honeybees. These insects meet their nutritional requirements from phloem, and their digestive by-products are collected by honeybees (Ülgentürk et al., 2013). Honeydew honeys possess different characteristics to floral

Table 3
Some sugars and mineral values of different floral honeys.*

	Monosaccharide%		Disaccharide%				Trisaccharide%	F/G	F + G
	Fructose	Glucose	Sucrose	Maltose	Trehalose	Melibiose	Melezitose		
Chestnut	38.44 ± 2.72	19.35 ± 3.00	1.02 ± 0.58	0.53 ± 0.12	0.09 ± 0.01	0.40 ± 0.40	0.56 ± 0.22	1.98	57.79
Astragalus	32.62 ± 10.17	25.34 ± 7.62	1.61 ± 0.08	0.16 ± 0.70	N.D.	0.36 ± 0.07	0.65 ± 0.47	1.28	57.96
Heather	45.11 ± 0.24	25.00 ± 0.32	N.D.	0.31 ± 0.03	0.04 ± 0.001	0.39 ± 0.14	0.55 ± 0.01	1.80	70.11
Clover	42.47 ± 2.00	17.40 ± 3.26	N.D.	N.D.	N.D.	0.37 ± 0.01	0.55 ± 0.01	2.44	59.87
Lavender	32.65 ± 1.28	22.19 ± 6.79	N.D.	0.36 ± 0.03	N.D.	0.36 ± 0.03	0.52 ± 0.01	1.47	54.84
Lime	44.12 ± 2.13	23.64 ± 1.20	N.D.	0.13 ± 0.04	N.D.	N.D.	0.43 ± 0.02	1.86	67.76
Jerusalem tea	40.16 ± 2.79	22.30 ± 1.11	N.D.	0.18 ± 0.56	0.10 ± 0.05	0.36 ± 0.01	0.56 ± 0.09	1.80	62.46
Common eryngo	41.85 ± 5.66	21.83 ± 0.70	N.D.	0.38 ± 0.01	N.D.	N.D.	0.50 ± 0.10	1.91	63.68
Chaste tree	37.10 ± 3,96	25,90 ± 1,32	N.D.	N.D.	N.D.	N.D.	0.57 ± 0.08	1.43	63.00
Rhododendron	43.58 ± 2.05	23.16 ± 4.19	N.D.	0.96 ± 0.01	0.02 ± 0.01	0.36 ± 0.02	0.54 ± 0.01	1.88	66.74
Oak	43.28 ± 2.18	21.73 ± 2.40	N.D.	0.19 ± 0.44	0.42 ± 0.12	N.D.	0.94 ± 0.07	1.99	65.01
Pine	39.80 ± 2.84	23.67 ± 2.21	N.D.	0.54 ± 0.64	0.23 ± 0.10	0.35 ± 0.01	0.62 ± 0.34	1.68	63.47
Acacia	28.30 ± 3.16	24.16 ± 2.76	3.39 ± 1.20	0.12 ± 0.50	N.D.	0.86 ± 0,04	0.61 ± 0.05	1.17	52.46
Multifloral	32.35 ± 5.65	25.07 ± 6.59	0.91 ± 0.16	1.05 ± 0.87	N.D.	N.D.	0.52 ± 0.04	1.29	57.42

N.D., not detected.
* Ribose, arabinose and galactose were not detected.

Table 4
Antioxidant properties of Turkish honey samples.*

	Total phenolic (mg GAE/100 g)	Total flavonoid (mg QE/100 g)	FRAP (μmol $FeSO_4{\cdot}7H_2O$/g)	DPPH SC_{50} (mg/mL)
Chestnut	98.26 ± 17.77^a	8.10 ± 2.56^a	4.30 ± 0.13^a	20.05 ± 5.42^b
Astragalus	43.63 ± 20.66^b	0.86 ± 0.49^c	0.66 ± 0.74c	123.56 ± 25.12^c
Heather	105.46 ± 26.08^a	5.84 ± 1.80^b	1.42 ± 0.28^c	27.84 ± 13.20^b
Clover	25.53 ± 5.90^c	0.65 ± 0.42^c	0.59 ± 0.21^c	98.19 ± 58.03^b
Lavender	53.39 ± 23.34^b	2.20 ± 1.54^c	0.67 ± 0.25c	70.20 ± 31.50^b
Lime	41.20 ± 4.10^b	0.95 ± 0.18^c	0.86 ± 0.12^c	76.20 ± 12.30^b
Jerusalem tea	40.17 ± 14.10^b	2.80 ± 1.10^c	0.65 ± 0.46^c	61.05 ± 5.20^b
Common eryngo	40.46 ± 14.10^b	0.85 ± 0.64^c	2.27 ± 0.96^c	60.08 ± 6.10^b
Chaste tree	35.60 ± 4.04^c	0.95 ± 0.24^c	0.67 ± 0.11^c	121.05 ± 20.40^c
Rhododendron	23.55 ± 10.22^c	0.92 ± 0.39^c	0.67 ± 0.22^c	78.06 ± 28.65^b
Oak	120.04 ± 18.56^a	3.10 ± 0.56^b	3.07 ± 0.84^a	12.56 ± 2.50^a
Pine	61.42 ± 5.59^b	1.58 ± 1.30^c	1.48 ± 0.83^c	44.30 ± 25.07^b
Acacia	16.02 ± 2.70^d	1.58 ± 0.22^c	0.64 ± 0.34^c	152.40 ± 62.00^c
Multifloral	29.54 ± 12.71^d	1.65 ± 0.80^c	0.79 ± 0.62^c	68.00 ± 20.50^b

Different letters (a–d) in the same columns are significantly different at the 5% level ($p < 0.05$).
* Means ± standard deviations.

honeys in terms of some physical and biological active properties, and these are found in very different types, such as pine, oak, willow, abies and picea honey (Ülgentürk et al., 2013). Pine honey is the best known honeydew honey, the majority of which is produced in Turkey and Eastern Europe (González-Paramás et al., 2007). Two different honeydew honeys were used in this study, oak and pine. No significant marker has to date been described for differentiating the honeydew honeys from one another (González-Paramás et al., 2007).

The origins of the honeydew honeys in the study were therefore based on the floral sources of the regions in which the honeys were produced and on statements by the beekeepers. In contrast to pine honey, oak honey can be produced in two ways. Pine honey is produced from a secretion resulting from absorption of pine phloem by female members of *Marchalina hellenica* (Sternorhyncha: Coccoidea, Marchalinidae) (González-Paramás et al., 2007; Ülgentürk et al., 2013). Oak honey, however, is the result of the collection of sugary substances that seep out of oak under various stress conditions (moisture and temperature) or from digestive by-products of various aphids found on oak tree leaves (*Quercus* sps. L.). The northwest of Turkey is rich in oak forests (*Q. robur*, *petraea*, *frainette*, and *hartwissiana*), while the southwest has abundant pine forests (*P. brutia*, *pinea*, and *halepensis*), both regions being ideal for honey production (Ülgentürk et al., 2013). We determined that oak honey is darker in color than pine honey and has much higher total phenolic contents and antioxidant capacity. Previous studies have reported that honeys from various species of oak (holm oak and Pyrenean oak) are dark-colored and contain high phenolic substances and antioxidant properties in association with this (González-Paramás et al., 2007). One study reported TPC levels of between 73 and 127 mg GAE/100 g from honeydew honeys collected from various regions of Romania (Bobiş, Mărghitaş, Dezmirean, Chirilă, & Moritz, 2011).

The floral honeys with the highest phenolic contents in this study were heather and chestnut. Chestnut and heather honeys have also previously been reported to possess high phenolic contents and high antioxidant properties in association with this (Alissandrakis et al., 2011; Küçük et al., 2007; Tezcan et al., 2011). *In vivo* and *in vitro* studies have shown that chestnut honey possesses many active biological characteristics, such as hepatoprotective, antiulcerogenic and monoamino-oxidase inhibiting effects (Nasuti et al., 2006; Yıldız et al., 2013). In this study, floral and light-colored honeys such as acacia, rhododendron, lime and clover exhibited low phenolic contents. Similar to our findings, other studies have also reported that multifloral honeys are light-colored and have low phenolic contents (Alves, Ramos, Gonçalves, Bernardo, & Mendes, 2013; Tezcan et al., 2011). One study reported that the total phenolic contents of honeys collected from various regions of Portugal varied between 115 and 140 mg GAE/100 g and that dark-colored honeys had higher phenolic contents (Alves et al., 2013). Other studies have also reported that heather honey is dark-colored with total phenolic contents of 72.7 mg/GAE/100 g, and that light-colored honeys' TPC range between 22 and 40 mg GAE/100 g (Ferreira, Aires, Barreira, & Estevinho, 2009).

Various floral honeys are regarded as medicinal honeys with high polyphenol contents. Manuka is one such honey, and is produced from Manuka plants (*L. scoparium* and *polygalifolium*). The level of phenolic contents of Manuka, a dark-colored honey, varies depending on the species of Manuka involved and the region where it is produced. For example, the phenolic contents of Manuka honeys from northern regions of New Zealand vary across a wide spectrum (90–270 mg GAE/100 g) (Jonathan et al., 2010). Another study reported total phenolic contents of 89.90 mg GAE/100 g for Manuka honey (*L. scoparium*) (Alzahrani et al., 2012). The honeys with the polyphenol values closest to those of Manuka honey in our study were oak, chestnut and heather.

3.4. Antioxidant properties

Flavonoids are an important sub-branch of the polyphenol family, synthesized by plants with the capacity to produce countless aromatic and phenolic compounds. In this study, the total flavanoids (TFC) of honeys collected from different areas of Turkey ranged between 0.65 and 8.10 mg quercetin per 100 g honey (Table 4). The differences between the different honey types were significant ($p < 0.05$). Flavonoids represented 2–10% of the total phenolic contents of the honeys investigated. Chestnut honey samples exhibited the highest amount of TFC (8.10 mg QUE/100 g), and clover and common eryngo honeys the lowest (0.63 mg QUE/100 g; 0.75 mg QUE/100 g). One study of Polish honeys reported that flavonoid levels represented approximately 5% of TPC (Jasicka-Misiak, Poliwoda, Dereń, & Kafarski, 2012). Although acacia had the lowest TPC, it had a higher level of flavonoids than many light-colored honeys (common eryngo, clover, Jerusalem tea, lime etc.). These results show that the phenolic compositions of honey are affected by floral sources.

Molecules that prevent oxidation in living organisms and that reduce or completely eradicate already formed oxidation are known as antioxidants. Due to the secondary metabolites it contains, honey is a good antioxidant compound, while honey's antioxidant capacities vary depending on species (Alves et al., 2013; Küçük et al., 2007; Tezcan et al., 2011). In this study, honeys' antioxidant capacities were determined using phenolic and total flavonoid contents as well as ferric reducing antioxidant capacity (FRAP) and DPPH radical scavenging activity tests. The total ferric reducing activities of all honey samples were correlated with total phenolic contents and DPPH radical scavenging activities (r^2: 0.81 $p < 0.05$, r^2: 0.70). Chestnut and oak honeys had the highest FRAP values, followed by heather, pine and Jerusalem tea honeys. Light-colored honeys had lower FRAP values than dark-colored honeys. In general, higher TPC indicates higher antioxidant capacity of honey samples as well as FRAP values (Alves et al., 2013; Ferreira et al., 2009; Tezcan et al., 2011). One study reported that dark-colored Portuguese honeys had higher antioxidant activities than light-colored honeys (Estevinho, Pereira, Moreira, Dias, & Pereira, 2008). However, Jerusalem tea honey had higher FRAP values than some light-colored honeys (Jerusalem tea, clover, rhododendron and lime). This may be attributed to the different phenolics present in the samples. All the phenolic compounds in honey samples, such as gallic acid, ferulic acid, rutin, quercetin and apigenin, have different reducing potentials. The reducing power of ferric tripyridyltriazine (Fe-III-TPTZ) complex determines total antioxidant capacity, and the reducing capacity is the sum of the reducing powers of the individual phenolic compounds present in honeys (Küçük et al., 2007). Previous studies have reported that dark-colored honeys have higher antioxidant activities (Alves et al., 2013; Alzahrani et al., 2012; Bertoncelj et al., 2007; Jasicka-Misiak et al., 2012; Tezcan et al., 2011).

3.5. Phenolic profiles

Eighteen standard phenolic standards were analyzed qualitatively and quantitatively using RP-HPLC-UV (Table 5). *p*-OH benzoic, caffeic and *p*-coumaric acid were present in differing amounts in all the unifloral honeys investigated, while chlorogenic acid, myricetin and fisetin were not detected in any honeys. The highest levels of *p*-OH benzoic acid were detected in oak and lime honeys. The highest levels of gallic acid were determined in oak honey, and the highest level of protocatechuic acid in pine honey. Especially, chestnut honey was rich in protocatechuic and *p*-OH benzoic acid. Although chestnut honey had the highest total phenolic compounds, the analyzed phenolic compounds were lower than expected (Tables 4 and 5). This shows that there may be

Table 5
Phenolic compounds detected with HPLC-UV of Turkish honey species (mean ± SD measured as μg/g samples).*

Compound	Chestnut	Astragalus	Heather	Clover	Lavender	Lime	Jerusalem tea	Common eryngo	Chaste tree	Rhododendron	Oak	Pine	Acacia	Multifloral
Gallic acid	0.91 ± 0.29	0.29 ± 0.20	0.61 ± 0.01	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.09 ± 0.08	82.49 ± 4.38	N.D.	N.D.	N.D.
Protocatechuic acid	7.21 ± 1.67	5.082 ± 3.84	19.43 ± 2.92	2.75 ± 0.03	N.D.	9.24 ± 0.24	N.D.	N.D.	N.D.	1.95 ± 1.17	744.60 ± 105.41	81.19 ± 713	N.D.	N.D.
p-OH benzoic acid	13.57 ± 5.50	33.19 ± 22.36	25.42 ± 2.08	15.80 ± 6.88	24.02 ± 3.00	55.26 ± 1.11	17.84 ± 3.80	26.69 ± 6.43	24.68 ± 0.19	10.93 ± 5.4	50.191 ± 36.21	29.24 ± 18.57	11.59 ± 0.35	19.71 ± 17.40
Catechin	N.D.	N.D.	23.07 ± 0.26	N.D.	7.85 ± 1.38	N.D.	N.D.	17.10 ± 0.23	1.87 ± 0.30	N.D.	N.D.	21.75 ± 15.30	N.D.	N.D.
Chlorogenic acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Vanillic acid	1.95 ± 0.77	1.67 ± 0.20	N.D.	N.D.	N.D.	N.D.	9.47 ± 7.43	N.D.	N.D.	1.40 ± 0.96	N.D.	20.00 ± 0.22	N.D.	N.D.
Caffeic acid	4.83 ± 3.25	5.14 ± 4.03	0.76 ± 1.50	1.51 ± 0.39	3.88 ± 2.73	8.80 ± 1.01	6.60 ± 5.09	6.18 ± 2.15	3.67 ± 1.40	3.43 ± 2.01	26.78 ± 9.14	3.90 ± 3.39	1.35 ± 0.07	2.03 ± 16.72
Syringic acid	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	18.78 ± 5.58	4.59 ± 0.12	18.14 ± 3.98	16.10 ± 6.11
Epicatechin	N.D.	N.D.	N.D.	N.D.	28.40 ± 0.46	N.D.	N.D.	N.D.	N.D.	N.D.	10.32 ± 0.51	N.D.	N.D.	N.D.
p-Coumaric acid	5.52 ± 3.26	5.08 ± 3.68	2.98 ± 1.60	N.D.	2.56 ± 1.11	4.66 ± 1.11	3.91 ± 0.79	6.02 ± 0.39	2.21 ± 0.46	4.62 ± 1.21	15.95 ± 2.22	4.41 ± 3.46	1.99 ± 1.01	7.26 ± 4.32
Ferulic acid	1.64 ± 0.70	0.94 ± 0.59	N.D.	N.D.	N.D.	N.D.	0.88 ± 0.24	1.93 ± 0.09	0.36 ± 0.23	1.25 ± 1.01	4.19 ± 3.00	2.14 ± 1.30	0.97 ± 0.37	9.35 ± 4.98
Rutin	N.D.	4.61 ± 0.62	N.D.	N.D.	11.90 ± 1.34	N.D.	N.D.	N.D.	N.D.	N.D.	538.62 ± 124.20	11.64 ± 6.92	N.D.	7.48 ± 3.97
Quercetin	3.500 ± 0.07	N.D.	21.05 ± 0.512	N.D.	N.D.	N.D.	4.90 ± 1.07	N.D.	N.D.	3.63 ± 0.45	N.D.	11.77 ± 2.32	N.D.	N.D.
Apigenin	0.61 ± 0.44	0.61 ± 0.44	N.D.	N.D.	N.D.	34.83 ± 9.80	3.03 ± 0.24	N.D.	1.84 ± 0.49	0.53 ± 0.12	N.D.	N.D.	3.59 ± 0.01	N.D.
Kaempferol	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.19 ± 0.28	N.D.	7.50 ± 0.11	0.44 ± 0.13	N.D.
Isorhamnetin	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	3.25 ± 0.432	N.D.

N.D., not detected.
* Myricetin and fisetin were not detected.

another phenolic compound(s) in chestnut honey than the 18 phenolics investigated by HPLC-UV. The honey richest in catechin was heather, and oak and lavender honeys were rich in epicatechin. Isorhamnetin was determined only, albeit in significant amounts, in acacia honey, but was not present in any other honey. The highest levels of quercetin were determined in heather and pine honey, but none was detected in oak honey. Significant amounts of apigenin were present in lime honey, while lower levels were detected in acacia, Jerusalem tea, astragalus, chaste tree and chestnut. The highest rutin was detected in oak honey (538.62 μg/100 g) and the lowest levels in lavender honey (11.90 mg/g), pine (11.64 mg/g) and astragalus (4.61 mg/g) honeys. Major levels of *p*-OH benzoic acid were present in rhododendron honey, while lower levels of *p*-coumaric acid, quercetin, caffeic acid, kaempferol and apigenin were detected.

The major phenolic compound in lime honey was *p*-OH benzoic acid, and lower levels of apigenin, protocatechuic acid and *p*-coumaric acid were also detected. Caffeic acid was present in significant amounts in oak honey (11.78 mg/g), followed by Jerusalem tea and chestnut honey. The phenolic compositions of oak and pine, both honeydew honeys, differed considerably from one another. The major phenolic compounds in oak honey were protocatechuic, gallic and *p*-OH benzoic acid, but no gallic acid was determined in pine honey. In addition, rutin, catechin, quercetin and kaempferol were determined in pine honey, but not in oak honey. The hightest levels of protocatechuic acid were determined in honeydew honeys. Higher levels were detected in oak honey (744.60 mg/g) than in pine honey. Gallic acid can be used as a marker in differentiating these two honeydew honeys. The presence of quercetin and kaempferol has been reported in previous studies, in agreement with our own (Karabagias, Vavoura, Badeka, Kontakos, & Kontominas, 2014), together with absence of gallic acid (Tsiapara et al., 2009).

The major compound in acacia honey was syringic acid, and apigenin, kaempferol and isorhamnetin flavonoids were specifically determined in this study. In addition to acacia honey, it was also determined in multifloral honeys and at very low levels in pine honey, but not in other honeys. Syringic acid may be a marker for acacia honey (Kečkeš et al., 2013). Methyl syringate and syringic acid isomers are also important phenolic compounds in Manuka honey, known for its high antioxidant activities (Stephens et al., 2010).

Similarities and differences have been reported in terms of phenolic composition between Polish heather honeys and other heather honeys (Jasicka-Misiak et al., 2012). For example, *p*-OH benzoic acid, quercetin, coumaric acid, and caffeic acid have been determined in both heather honeys, while chlorogenic acid, vanillic acid and ferulic acid have only been identified in Polish honeys (Jasicka-Misiak et al., 2012). One earlier study reported that heather honeys from Portugal contained a considerable amount of *p*-coumaric acids, and smaller amounts of *p*-OH benzoic acid, syringic acid, and ellagic acid (Andrade et al., 1997). Comparing our results with those of that study, *p*-OH benzoic acid, protocatechuic acid, and catechin were major phenolic compounds, while syringic acid was not detected. Although there were some similar results due to the dominant pollen feature of the honey types, full compatibility could not be guaranteed. This means that honeys' phenolic compositions do not depend on plant flora alone, and that they also vary in line with the geographic characteristics of the region of production (Mendes et al., 1998).

In conclusion, this study revealed that Turkey, with its rich plant flora, is also rich in terms of honey diversity. Several types of honey of monofloral origin were compared in this study in addition to multifloral honeys, which investigate the physical, chemical, and antioxidant characteristics of 13 different monofloral honeys from Turkey. The sugar, phenolic compounds, and

antioxidant properties of the honeydew honeys pine and oak honeys differed from those of other types of honey. In addition to honeydew honey, chestnut and heather honeys had higher electrical conductivity, phenolic compounds and, associated with that, antioxidant properties than other floral honeys.

Although the great majority of honey characteristics were compatible with the honey index data, others remained outside those limits. These up to date data suggest that honeys of Turkish origin can represent a basis for revision of the honey indices. It also emerged that the true quality of honey cannot be completely accounted for with honey codex data and that the total polyphenol content is a very significant parameter. Finally, the physicochemical and biologically active properties of honey are affected by the flora from which it is produced and by geographical variations.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

This project was supported by the Turkish Ministry of Food and Agriculture (TAGEM-AR-GE/15). The authors are also grateful to all beekeepers and who assisted with the collection of honey specimens.

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