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ORIGINAL PAPER

Physicochemical properties, total phenolic content, and antioxidant activity of chestnut, rhododendron, acacia and multifloral honey

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Abstract

The aim of this study is to investigate the physicochemical properties, antioxidant activities, and total phenolic contents of the plateau (multifloral), chestnut, rhododendron, and acacia honey collected from Ordu province in Turkey. The rhododendron honey had the highest acidity ($p < 0.05$) while the chestnut honey showed the highest conductivity (1.13 ± 0.25 mS/cm) among all ($p < 0.05$). The highest diastase activities and the lowest HMF contents were determined in the multifloral and chestnut honey. Considering the DPPH and FRAP assays, the multifloral honey showed the highest antioxidant activity. Accordingly, the highest total phenolic content was determined in the multifloral honey followed by rhododendron, chestnut, and acacia honey. A moderate correlation was determined between the total phenolic content and antioxidant activities ($r = 0.575$ for FRAP and $r = 0.697$ for DPPH). Consequently, the plateau honey could be recommended for nutritional and health purposes due to its relatively higher antioxidant activity and total phenolic content together with relatively lower HMF content and higher diastase activity. Considering that Ordu is the largest honey producer city in Turkey, the second top honey producer country, this work could shed light for future studies and be taken as a reference providing insights on the characteristics of different types of honey for both local and worldwide producers.

Keywords Diastase · DPPH · FRAP · HMF · Plateau honey · Proline

Introduction

Honey is the most consumed bee product around the world for nutritional and curative purposes among the valuable arts that honeybees (Apis spp.) generate including propolis, pollen, royal jelly, beeswax, honeybee venom, and bee bread (perga). Honey contains approximately 200 identified compounds with a typical composition of 38% fructose, 31% glucose,10% other sugar types, 18% water, and 3% other substances. However, this 3% is considered as the most important portion of honey residing valuable components such as carotenoids and phenolics [1, 2].

Due to its therapeutic features, there are many studies available on the beneficial effects of honey on human biological processes including antioxidant [1, 3], antimicrobial [4–6], wound healing [7, 8], antidiabetic [9, 10], anti-inflammatory [11, 12], and anticancer [13, 14] activities. Furthermore, owing to its antiviral effects [15], honey has been applied in clinical trials in the USA and Egypt hoping to discover potential curing effects against the current COVID-19 pandemic [16]. Most of these benefits of honey are attributed to its unique phenolic composition which depends mainly on seasonal and environmental factors [17]. Phenolic profiles of different honey types were determined by Kivrak et al. [18] and Can et al. [19]. For example, the main phenolic compounds of the rhododendron honey were determined as benzoic acid derivatives, coumaric, caffeic, and ferulic acids whereas benzoic acid derivatives, vanillic, protocatechuic, and coumaric acids were the ones detected in the chestnut honey.

Climatic conditions and geographical location makes Turkey a favorable country for honey production. Therefore, it is home to both unifloral and multifloral honey from nectar and honeydew. According to statistical data, Ordu, harboring 573.358 hives, is the largest honey-producer city of Turkey with an annual honey production of 17.057 tons [20]. However, the information on essential physical, chemical,

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and biological properties of honey from this region is limited. Honey characteristics have been shown to depend on the nectar type as well as the region [21, 22]. Therefore, the purpose of this study is to evaluate the physicochemical and biochemical properties of four different types of honey including chestnut, acacia, rhododendron, and a multifloral (locally known as plateau honey) honey collected from the hives located in Ordu province.

Materials and methods

Honey samples

Honey samples, consisted of four different types, were supplied by three different local beekeepers (12 samples in total) from the regions and approximate coordinates indicated in the brackets as follows: namely chestnut honey (Gurgentepe: 40.78951, 37.60017), acacia (Altınordu: 40.98554, 37.87924), rhododendron (Korgan: 40.82925, 37.34412), and multifloral (known as plateau honey among locals) honey (Mesudiye: 40.446381, 37.77368) (Fig. 1). To avoid the temperature effect, the samples were stored at room temperature away from sunlight until analyses.

Moisture content

The moisture content was determined using the refractometric method suggested by Bogdanov [23]. Briefly, a homogenized honey sample was put in a closed container and placed in a water bath set to 50 ± 0.5 °C to allow all the sugar crystals to be dissolved prior to the analysis. The sample was then cooled to room temperature and stirred again. The refractive indices of honey samples were read using a refractometer (RX-50000X, Atago, Japan) at 20 °C and corresponding moisture contents were recorded [24].

Free acidity

Titrimetric analysis was performed to determine free acidity following the method given by Bogdanov [23]. Basically, the acidity was calculated based on the volume of consumed 0.1 M NaOH for titration of the diluted honey sample (10 g:75 mL distilled water) to reach a pH of 8.3 and the results were expressed as milliequivalents per kg of honey.

Electrical conductivity (EC)

A conductivity meter (H1763100, Edge, Hanna Instruments Inc., RI, USA) was used for EC measurements of honey samples [20% (w/v) in distilled water]. The cell constant was determined using 0.1 M potassium chloride solution. All measurements were performed at 20 °C in triplicate, and the means are expressed as MilliSiemens per centimeter (mS/cm).

Diastase activity

The diastase activity of honey samples was determined following the Phadebas method according to the procedure of Bogdanov [23] with small modifications. Honey sample (1 g) was dissolved in acetate buffer to a final volume of 100 mL. Then, 5 mL of this solution was transferred to a test tube and placed in a water bath at 40 °C for 5 min. A Phadebas tablet (Pharmacia Diagnostics AB, Uppsala, Sweden) was added to the solution and vortexed for 10 s. The solution was transferred back to the water bath and incubated for 30 min. The reaction was terminated by adding 1 mL

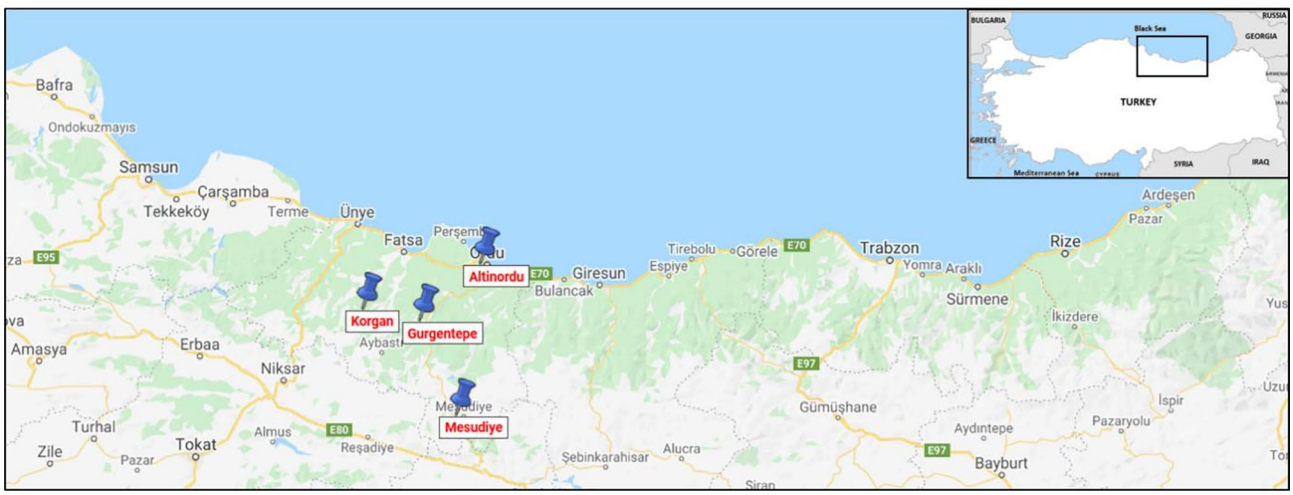

Fig. 1 Locations of collected honey samples

of 0.5 M NaOH flowed by vortexing for 5 s. The solutions were centrifuged (3662×*g* for 5 min) and absorbances were recorded at 620 nm wavelength against blank. The diastase activity was calculated from the absorbance readings and expressed in Schade units using Eqs. 1 and 2 given below [25].

$$\text{Diastase activity (as Schade unit)} = 28.2 \times \Delta A620 + 2.64 \,(\text{if } 8;\text{diastase units};40) \quad (1)$$

$$\text{Diastase activity (as Schade unit)} = 35.2 \times \Delta A620 - 0.46 \,(\text{if diastase units} \leq 8) \quad (2)$$

Hydroxymethylfurfural (HMF) content

HMF content was determined using a reverse-phase HPLC equipped with UV detection system and a C18 column (ODS Hypersil C18, Thermo Scientific, Inc., MA, USA) as explained by Bogdanov [23]. The method is based on the dissolution of honey in water (10 g to a final volume of 50 mL) and its filtration through a membrane filter (0.45 µm) prior to injection to the HPLC system and signal detection at 285 nm wavelength.

Proline content

Proline content was determined according to the official TS 13357 method [26]. The method involves the formation of a colored complex as a result of the reaction between proline and ninhydrin and spectrophotometric measurement of the color intensity at 510 nm wavelength. The proline content is expressed as mg proline/kg honey.

DPPH (1,1-diphenyl-2-picrylhydrazyl) assay

The DPPH radical scavenging activity of honey samples was determined as a measure of antioxidant activity according to Bergamo et al. [27] with some modifications. Briefly, each honey sample (2 g) was dissolved in 10 mL of methanol using a vortex-mixer. An aliquot of this solution (3 mL) was mixed with a freshly prepared DPPH solution (0.1 mM in 80% methanol). The mixture was kept in the dark at room temperature for 30 min and absorbance values at 517 nm were recorded. All readings were made in triplicate and results are expressed as the inhibition values [Inhibition (%) $= 100 \times (Abs_{blank} - Abs_{sample})/Abs_{blank}$] [28].

Ferric ions (Fe^{3+}) reducing antioxidant power assay (FRAP)

The reducing powers of samples were determined by the method of Gülçin et al. [29]. Briefly, a 240 µL sample solution, from the stock honey sample solutions prepared for DPPH assay, was mixed with sodium phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min followed by the addition of 2.5 mL of trichloroacetic acid (10%). Then, this solution was mixed with distilled water (2.5 mL each) and 0.5 mL of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm using a spectrophotometer. Trolox was used as the standard compound, all readings were made in triplicate and the antioxidant activity is expressed as mg trolox equivalent (TE)/g honey.

Total phenolic content (TPC)

The total phenolic content of the honey samples was determined with the Folin–Ciocalteu method as described by Gülçin et al. [29]. A 1 mL aliquot of honey stock solution was diluted with distilled water (46 mL), then 1 mL of Folin-Ciocalteu reagent was added and mixed thoroughly. Three minutes later, 3 mL of Na_2CO_3 (2%) was added and the mixture was diluted to a final volume of 50 mL, followed by incubation at room temperature in the dark for 2 h with intermittent shaking. The absorbance readings at 760 nm were recorded. A standard curve was plotted using gallic acid to calculate the concentration of total phenolic compounds in the honey (mg gallic acid equivalent (GAE)/g honey).

Statistical analysis

All analyses were performed in triplicate. Statistical analyses were performed using Minitab 17 (Minitab Inc., State College, PA, USA). One-way analysis of variance (ANOVA) through the general linear model was applied ($\alpha = 0.05$). For comparison, significant differences ($p < 0.05$) between the means were further analyzed using the Tukey Test. All values were normally distributed ($p > 0.05$) according to the Anderson–Darling normality test hence the Pearson's correlation coefficient was applied to understand the relationship between TPC, DPPH, and FRAP results.

Results and discussion

Moisture

The moisture content is an indicator of the stability and shelf life of honey. The lower the moisture content, due to less favorable conditions for fermentation and spoilage by microorganisms, the higher the stability and resistance to granulation of honey is. Also, it gives an idea on the botanical and geographical origin of the nectar, climatic conditions, the season of harvesting, adulteration by sellers/producers, processing and storage conditions [30, 31]. It is recommended that honey should be stored at 10–16 °C under a relative humidity less than 65% in a hermetically sealed container to minimize moisture capture from the environment [32].

The moisture content of honey samples ranged between 14.45 and 21.62% with an average value of 18.43 ± 1.82% (Table 1). This value is in compliance with the regulations of TFC [33] and EU [34] which limited the moisture content to 20% at maximum. The highest and the lowest moisture contents were determined in the rhododendron (18.89 ± 1.16%) and acacia (17.99 ± 3.11%) honey, respectively. However, the moisture content was not affected significantly by honey type ($p = 0.789$) and there was no significant difference between honey types in terms of moisture content. These moisture contents are similar to the ones obtained in the studies by Kivrak et al. [18], Šarić et al. [35], and Zappala et al. [36] for chestnut, rhododendron, and acacia honey.

Table 1 Physicochemical characteristics of honey samples

Parameters	Honey type	Mean value ± SD	Min	Max
Moisture (%)	Multifloral honey	18.39 ± 1.30[a]	17.48	20.12
	Chestnut	18.45 ± 1.17[a]	17.38	19.96
	Acacia	17.99 ± 3.11[a]	14.45	21.62
	Rhododendron	18.89 ± 1.16[a]	17.92	20.42
	$\bar{X}$±SD	18.43 ± 1.82	14.45	21.62
Free Acidity (meq/kg)	Multifloral honey	24.67 ± 4.61[b]	19	31
	Chestnut	17.33 ± 5.15[b]	12	25
	Acacia	16.33 ± 3.00[b]	12	21
	Rhododendron	34.33 ± 13.04[a]	16	44
	$\bar{X}$±SD	23.17 ± 10.26	12	44
Conductivity (mS/cm)	Multifloral honey	0.20 ± 0.00[b]	0.20	0.21
	Chestnut	1.13 ± 0.25[a]	0.80	1.31
	Acacia	0.19 ± 0.06[b]	0.14	0.27
	Rhododendron	0.32 ± 0.03[b]	0.28	0.36
	$\bar{X}$±SD	0.46 ± 0.42	0.14	1.31

Mean values in the same column followed by different uppercase letters are significantly different ($p < 0.05$)

SD standard deviation

Free acidity

Acidity is an important criterion that affects the organoleptic and physical properties such as color and conductivity. More than 30 different acids were identified in honey. Although nectar is a source of organic acids (citric, malic, oxalic), the majority of the acid is generated through the enzymatic activity of bees e.g. gluconic acid which is formed through break down of glucose by the enzyme glucose oxidase. The type of organic acids present in honey is important since the dominant organic acid may give hints about the botanical origin of the honey [37]. Furthermore, high acidity is usually associated with dark-colored honey [31]. The free acidity values of the honey samples are given in Table 1. The honey type affected the acidity levels of honey samples significantly ($p < 0.001$). The average acidity was 23.17 ± 10.26 meq/kg while the rhododendron and multifloral honey had the highest acidities, respectively. The rhododendron honey had a significantly higher acidity level than its counterparts ($p < 0.05$), whereas, there was no significant difference between acacia, chestnut, and multifloral honey regarding the acidity ($p > 0.05$). These values are under the limit of 50 meq/kg honey set by both EU [34] and TFC [33]. Considering these results, the relatively low acidity of chestnut is reasonable since it is known to have higher pH (5–6) hence less acidity compared to other blossom honey which usually have a pH value in the range of 3.3 to 4.6 [23]. Similarly, Kivrak et al. [18] found that rhododendron honey had higher acidity with respect to chestnut and acacia honey, respectively. On the other hand, Küçük et al. [38] found that rhododendron had slightly lower acidity than chestnut. Although high acidity could protect the honey against microbial spoilage, it should be kept in mind that the presence of a high levels of acids, especially acetic acid, may also indicate spoilage by yeast fermentation.

Electrical conductivity (EC)

The EC values of honey samples are presented in Table 1. The honey type was found to be a significant factor affecting the EC value ($p < 0.001$). Among all, chestnut honey stands out regarding its EC value (1.13 ± 0.25 mS/cm) while the others have similar EC values (0.19–0.20 and 0.32 mS/cm). The EC value is directly proportional to the mineral and acid content of honey and has been commonly used to understand the botanical origin of honey. For example, although there are exceptions for certain types of honey, the EC of blossom honey must be lower than 0.8 mS/cm, on the contrary, chestnut and honeydew honey must have an EC higher than 0.8 mS/cm according to the EU [34]. Hence, these results are within the limits of the EU [34]. Similarly, Thrasyvoulou and Manikis [39] and Šarić et al. [35] determined an average of 1.54 and 1.27 mS/cm EC from Greek

and Croatian chestnut honey, respectively. Similar results were also obtained for chestnut, acacia, and rhododendron honey in different studies [18, 19, 36].

Diastase activity

As presented in Table 2, the honey samples presented a broad range of diastase number (4.00–39.3) and the effect of honey type on the diastase number was found significant ($p = 0.001$). The highest diastase activity was determined in the multifloral honey (26.17 ± 10.39). Diastase is one of the three main enzymes, together with invertase and glucose oxidase, found in honey. Enzyme activity, more specifically diastase activity, is highly dependent on environmental temperature. Moreover, together with HMF content, diastase activity is an indicator of freshness [40]. Storage under warm conditions and high-temperature treatments of honey would result in lower diastase activity. For example, it was reported that heating acacia honey (80 °C for 60 min) reduced diastase activity by around 20% [41]. The diastase half time is reported as 35 years, 4 years, and 200 days when stored at 10, 20, and 30 °C, respectively [32]. Therefore, this result is reasonable considering that multifloral honey is actually collected from high altitude plateaus which have relatively lower temperatures. Furthermore, plateaus can be conceived as slow nectar flux and less fertile environments enabling bees more time to process the nectar hence higher enzyme levels are observed in honey [31]. Acacia and rhododendron honey had significantly lower diastase activity compared to multifloral and chestnut honey ($p < 0.05$). Also, it could be speculated that acacia and rhododendron, as plants, may carry relatively less diastase since diastase in honey not only originates from the bee but also from the plants [42]. All honey samples possessed diastase numbers higher than 8 with an average of 18.10 ± 8.62 thereby complying with the standards [33, 34]. Similarly, Kivrak et al. [18] and Zappala et al. [36] determined that chestnut honey had higher diastase activity than acacia honey. However, diastase activity of rhododendron honey was found to be the highest followed by multifloral and chestnut honey (17.7), respectively in a study by Küçük et al. [38] while Can et al. [19] found that acacia honey had the highest diastase activity followed by rhododendron and chestnut respectively. These differences could be ascribed to climatic and floral differences of the regions where honey is harvested in different studies performed.

Table 2 Diastase activity, HMF and proline contents of honey samples

Parameters	Honey type	Mean value ± SD	Min	Max
Diastase number	Multifloral honey	26.17 ± 10.39^{a}	15.80	39.30
	Chestnut	21.10 ± 2.29^{ab}	19.10	24.10
	Acacia	13.67 ± 2.99^{bc}	10.80	17.50
	Rhododendron	11.47 ± 6.92^{c}	4.00	19.90
	$\bar{X}$±SD	18.10 ± 8.62	4.00	39.3
HMF (mg/kg)	Multifloral honey	3.64 ± 2.09^{c}	1.09	5.93
	Chestnut	1.59 ± 1.32^{c}	0.17	3.22
	Acacia	11.83 ± 4.17^{a}	8.31	17.34
	Rhododendron	8.02 ± 1.71^{b}	6.23	11.00
	$\bar{X}$±SD	6.27 ± 4.71	0.17	17.34
Proline (mg/kg)	Multifloral honey	692.67 ± 79.53^{a}	609	796
	Chestnut	758.56 ± 67.73^{a}	688	845
	Acacia	357.00 ± 34.38^{b}	312	393
	Rhododendron	535.00 ± 377.91^{ab}	204	1033
	$\bar{X}$±SD	585.81 ± 245.24	204	1033

Mean values in the same column followed by different uppercase letters are significantly different ($p < 0.05$)

SD standard deviation

Hydroxymethylfurfural (HMF) content

HMF is a chemical compound generated from sugar degradation, Maillard or caramelization, and its concentration is increased with heat treatment/exposure [43]. The HPLC method was found to be more appropriate for HMF determination in honey among the methods applied so far since, unlike UV-based methods, it does not interfere with the substances formed through heat and/or storage damage [36]. However, high HMF could also be found in honey harvested from warm regions [44]. Since fresh honey contains no or traces of HMF low HMF content is usually attributed to freshness [45, 46]. The HMF contents of honey samples are given in Table 2. The HMF content of honey was significantly affected by honey type ($p < 0.001$). The lowest HMF content was determined in the chestnut honey (1.59 ± 1.32 mg/kg) while the highest one was in the acacia honey (11.83 ± 4.17 mg/kg). In order for a kg of honey to reach to a level of 40 mg HMF, which is the highest level of HMF allowed by TFC [33] and EU [34], 2–4 years is required when stored at 20 °C Bogdanov [32]. Consequently, all honey samples possessed an HMF level inside the limits. Sakač et al. [47] determined an average of 2.57 mg/kg HMF concentration for 15 acacia honey collected from Serbia. Out of 17 Moroccan multifloral honey samples, HMF level was determined as 12.91 mg/kg in a study by Chakir et al. [48]. Can et al. [19] found that HMF content of acacia (12.56 mg/kg) was higher than those of chestnut (9.28 mg/kg) and rhododendron (3.20 mg/kg) honey, respectively. In another study, the HMF content of chestnut honey was determined to be higher than rhododendron and multifloral honey, respectively [38]. Honey types are lined up as

rhododendron > acacia > chestnut based on their HMF contents by Kivrak et al. [18]. As listed here, different types of honey are associated with high levels of HMF content in different studies. Therefore, it is difficult to make a statement for HMF contents of honey types unless they are harvested, processed, and stored under the same conditions.

Proline content

Proline is the most abundant amino acid (50–85% of total) out of the 26 identified in honey [49]. According to TFC [33], the minimum proline content must be 300 mg/kg in general. However, there are exceptional regulations for some unifloral honey types such as chestnut (≥ 500 mg/kg), acacia (≥ 120 mg/kg), and lavender (≥ 180 mg/kg) since proline content is also associated with its floral and botanical origin [50]. Considerably lower proline concentrations, as proline content decreases with storage, usually indicates ripeness and shows adulteration with sugar due to significantly lower proline content of syrup compared to nectars. Czipa et al. [41] showed that heating acacia honey for 60 min at 80 °C results in ≈10% loss of proline. Considering current results, all proline contents are found to be in compliance with the regulations of TFC [33] (Table 2). Honey type significantly affected the proline concentration of honey samples ($p = 0.001$). The highest proline levels were detected in the chestnut (758.56 ± 67.73 mg/kg) and multifloral (692.67 ± 79.53 mg/kg) honey, respectively while the lowest one was in acacia honey (357.00 ± 34.38 mg/kg). Accordingly, acacia honey was reported to have the lowest proline content among floral honey by Földházi et al. [51]. Similar results were obtained for acacia, chestnut, and rhododendron honey in different studies [18, 19, 35, 52, 53].

Antioxidant activity

Antioxidant activities of honey samples are presented in Table 3. Antioxidant activity was determined using both DPPH and FRAP which showed a weak to moderate correlation ($r = 0.371$). Although no significant difference was detected between antioxidant activities by the FRAP method ($p > 0.05$), multifloral honey had significantly higher antioxidant activity according to the DPPH method ($p < 0.05$). However, it cannot be ignored that the standard deviation values are quite high in almost all honey samples. Overall, it would be proper to mention that multifloral was the honey type possessing the highest antioxidant activity considering both test results. Similar rates were obtained in different studies for chestnut, multifloral, and acacia honey regarding FRAP activity [19, 38, 54]. Gül and Pehlivan [55] determined the DPPH radical scavenging activity of honey samples collected from Ordu as rhododendron > chestnut > acacia. Simultaneous collection of honey samples and storage under controlled conditions (temperature, humidity, light, etc.) are required to make a logical assessment of the antioxidant activity of honey samples [46].

Total phenolic content

Total phenolic contents varied greatly among the honey types (Table 3) and honey type affected the phenolic content significantly ($p < 0.05$). The lowest phenolic content

Table 3 Antioxidant activities and total phenolic contents of honey samples

Parameter	Honey samples	Mean value ± SD	Min	Max
FRAP activity (mg TE/g honey)	Multifloral honey	1.34 ± 0.51^{a}	0.64	1.81
	Chestnut	1.49 ± 0.14^{a}	1.28	1.63
	Acacia	0.68 ± 0.03^{a}	0.65	0.72
	Rhododendron	0.86 ± 0.22^{a}	0.57	1.17
	$\bar{X}$±SD	1.09 ± 0.44	0.57	1.81
DPPH activity (% inhibition)	Multifloral honey	30.68 ± 0.36^{a}	30.43	30.94
	Chestnut	2.51 ± 1.47^{b}	0.95	3.54
	Acacia	7.56 ± 5.02^{b}	2.35	11.97
	Rhododendron	11.67 ± 5.47^{b}	6.37	17.31
	$\bar{X}$±SD	11.09 ± 10.91	0.95	30.94
Total phenolic content (mg GAE/g)	Multifloral honey	0.26 ± 0.07^{a}	0.15	0.36
	Chestnut	0.12 ± 0.03^{bc}	0.09	0.16
	Acacia	0.02 ± 0.01^{c}	0.01	0.03
	Rhododendron	0.19 ± 0.06^{ab}	0.11	0.32
	$\bar{X}$±SD	0.15 ± 0.10	0.01	0.36

Mean values in the same column followed by different uppercase letters are significantly different ($p < 0.05$)

SD standard deviation, *TE* trolox equivalent

Table 4 Correlations between TPC and antioxidant activities of DPPH and FRAP

	FRAP activity	DPPH activity	TPC
FRAP activity			
r	1	0.371	0.575
p		0.235	0.05*
DPPH activity			
r	0.371	1	0.697
p	0.235		0.012
TPC			
r	0.575	0.697	1
p	0.05*	0.012*	

Asterisks (*) represent significant differences ($p < 0.05$)

r Pearson's correlation coefficient, *p* p value

was determined in acacia honey (0.02 ± 0.01) while the highest one was in multifloral honey (0.26 ± 0.07) with more than tenfold difference (Table 3). According to the phenolic contents, honey samples are ordered as mulfifloral > rhododendron > chestnut > acacia. Acacia honey was found to have the lowest TPC compared to chestnut and/or rhododendron honey in previous studies [18, 19, 54]. Czipa et al. [41], determined an average TPC of 0.17 mg/g from 44 Hungarian acacia honey. Parallel to the current results, an average TPC of 0.05 mg/g was determined in a study by Bertoncelj et al. [54] while averages of 0.16 mg/g and 0.19 mg/g were detected for acacia honey in the studies by Can et al. [19] and Kivrak et al. [18], respectively. Considering rhododendron honey, Silici et al. [56] found an average TPC of 0.21 mg/g from 7 honey samples collected from Ordu. In another study, chlorogenic acid and coumaric acid were found to be the main phenolic compounds in 12 rhododendron honey collected from the Black Sea region while ferulic acid was the most abundant phenolic in the honey ($n = 2$) collected from Ordu [57]. Kolayli et al. [58] determined the TPC of chestnut honey ($n = 15$) as 4.3 mg/g which is considerably higher than our results. Although Gül and Pehlivan [55] determined the TPC of rhododendron to be higher than chestnut, which is similar to the current findings, there are also studies in which the TPC of chestnut honey was determined to be higher than rhododendron [18, 38]. These differences could be attributed to seasonal and regional varieties of honey samples. Furthermore, correlation analysis indicated that TPC is in moderate correlation with antioxidant activities, both FRAP ($r = 0.575$) and DPPH ($r = 0.697$) (Table 4). The TPC and antioxidant activity usually show a good correlation [54, 59, 60] although there are studies indicating moderate correlation [61].

Conclusions

Unifloral honey such as chestnut, acacia, and rhododendron are accepted as higher quality honey due to their pure flavors while multifloral honey is a mixture of different flavors comprising its own unique flavor every time. Therefore, it is important to physicochemically characterize these honeys in order to identify their quality and purity together with possible adulteration. In compliance with former studies, this study gives a general idea of the properties of multifloral (plateau), chestnut, rhododendron, and acacia honey. Considering the previous studies it could be proper to say that although seasonal and regional differences affect the quality of each honey, all honey types have their unique characteristics. Further studies embracing sugar, phenolic, and aromatic profiles and thermal behaviors of honey are required for a deeper investigation and revelation of distinctive features.

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Author contributions LK designed the study, critically evaluated the results, and wrote the manuscript. NA performed the analyses using the selected methods and wrote the first draft of the manuscript. OFC contributed to the statistical evaluation of the data, the discussion of the findings, and the writing of the manuscript. All authors read and approved the final version of the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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