Evaluation of organic acid, saccharide composition and antioxidant properties of some authentic Turkish honeys

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Summary

Occurring alongside the main saccharide components, antioxidant compounds in honey play significant roles in human metabolism and nutrition. Six individual organic acids and two saccharides were determined by a simple and rapid capillary electrophoretic technique without any special treatment. Gluconic acid was the predominant organic acid and was detected in all samples. Formic, malic, citric and succinic acids were determined at minor concentrations, while oxalic acid was not detected in any of the samples. Glucose and fructose content was found between 223.50 $g \cdot kg^{-1}$ and 422.40 $g \cdot kg^{-1}$, and 310.20 $g \cdot kg^{-1}$ and 642.20 $g \cdot kg^{-1}$, respectively. The fructose/glucose ratios of the ten honey samples in this study were between 1.18 and 1.75 with an average value of 1.50. Gluconic acid content changed depending on the type of honey between 1.50 $g \cdot kg^{-1}$ and 13.8 $g \cdot kg^{-1}$ with an average value of 6.24 $g \cdot kg^{-1}$, while the other organic acids were at minor concentrations, or were not detected at all. The antioxidant properties of the honey were analysed in terms of total polyphenols, ferric-reducing/antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activities. Among the samples, darker honeys showed higher antioxidant values, related to their total phenolic compound contents.

Keywords

antioxidant; capillary electrophoresis; saccharides; honey; organic acids

Honey is a natural product consisting mainly of fructose and glucose, and the minor amount of other compounds like phenolics, proteins, enzymes, amino acids, minerals, vitamins, organic acids and Maillard reaction products, and possible other minor components [1]. Honey has been used in folk medicine since the early ages of human beings and, in recent times, its application in the treatment of burns, gastrointestinal disorders, asthma, infected wounds and skin ulcers has been re-investigated [2]. The antimicrobial action of honey has been known since the ancient times and honey has been used for long times for treating wounds. The composition, nutritional value, appearance and sensory properties of honey differ in relation to its botanical origin and geographic area where bee hives are located [3]. In recent years, honey is more and more important in our diet for its medicinal benefits. Many research works revealed that honey contains a variety of natural antioxidants. Due to health benefit effects of antioxidant compounds [4], there is an increasing number of research reports on the antioxidant capacities of honey samples from different origins and different regions [5–8]. Honeys contain a small proportion of organic acid (0.5%), which can be used as indicators of deterioration on account of storage, freshness, purity, and authenticity. Organic acids occur in honey as a result of aerobic and anaerobic fermentation. Organic acids are responsible for special flavours of honeys. Recently, researchers have drawn attention to the importance of organic acid profiles for description of honey quality and reviewed the current literature related to the significance of non-aromatic organic acids in honey and the analytical methods for the analysis of individual organic acids in honey samples [9]. It was suggested that, besides enzymatic methods and high performance liquid chromatography, capillary electrophoresis is a favourable method for the analysis of organic acids in honey samples. However, though there are lots of ap-

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plication reports on the quantification of organic acids in juices and beverages by capillary electrophoresis [10–13], surprisingly there exist only very few capillary electrophoretic reports published for the organic acid analysis of honey samples [14–15]. However, capillary electrophoresis besides its small sample consumption and short analysis times, is a very suitable method for the analysis of organic acids in samples containing high amounts of saccharides. Capillary electrophoresis is also easily used for the determination of saccharides in food products [16]. In the present study, we used capillary electrophoretic methods for the analysis of both organic acids and saccharide types of selected Turkish honey samples.

Turkey, which is the fourth largest honey producing country in the world, has a rare mix of suitable conditions for honey production like climate, topographical structure and richness of plant flora. Vegetation in the area is characterized by citrus, olive, pine, sunflower, thyme, chestnut, rhododendron and several mountain flowers. The composition, nutritional value, appearance and sensory properties of honey differ in relation to its botanical origin and geographic area where bee hives are located. Therefore, our research group intends to determine and compare, in some authentic Turkish honey samples from the Black sea region and one from West Anatolian region, major organic acid and saccharide composition, as well as total phenolic contents and antioxidant capacity.

MATERIALS AND METHODS

Chemicals and instrumentation

Citric acid monohydrate, 2,6-pyridinedicarboxylic acid, oxalic acid dihydrate, malic acid, *N*-cetyl-*N,N,N*-trimethylammonium bromide (CTAB), formic acid (d = 1.22 g·ml^{-1}, 98%), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and DPPH (1,1-diphenyl-2-picrylhydrazyl) were purchased from Merck (Darmstadt, Germany). TPTZ (2,4,6-tripyridyl-*s*-triazine), Folin-Ciocalteu's phenol reagent, and glycylglycine were from Fluka (Buchs, Switzerland). D(+)glucose and D(–)fructose, D-gluconic sodium salt, pyruvic acid and fumaric acid were from Sigma Chemical (Steinheim, Germany). Maleic acid, D-tartaric acid and succinic acid were from Supelco (Bellefonte, Pennsylvania, USA). BHT (butylated hydroxytoluene) was supplied by Applichem (Darmstadt, Germany).

Capillary electrophoretic separations were performed with an Agilent (Santa Clara, California, USA) capillary electrophoresis system equipped with a diode-array detector. Data processing was carried out with the Agilent ChemStation software. The fused silica capillary was 50 μm i.d., obtained from Polymicro Technologies (Phoenix, Arizona, USA). The total length of the capillary was 64 cm and the length to the detector was 56.5 cm. A new fused silica capillary was conditioned prior to use by rinsing with 1 mol·l^{-1} NaOH for 30 min and with water for 10 min. The capillary was flushed by 0.1 mol·l^{-1} NaOH and water for 2 min, and with buffer solution for 10 min in the beginning of every working day. Between runs, capillary was flushed for 2 min with the running buffer solution.

An ATI-Unicam UV-2 UV-VIS spectrophotometer (Cambridge, United Kingdom) was used for absorbance measurements. All solutions were prepared with deionized water purified in an Elgacan C114 (Elga, United Kingdom) filtration system.

Honey samples

The honey samples were directly obtained from the experienced beekeepers in the Black sea area of Turkey in 2007 flowering season. The honey samples differed in their colours as well as in their tastes. All the samples were stored at +4 °C until they were analysed. The colour index of the honey samples was measured as Pfund scale from the absorbance at 560 nm.

Preparation of the extracts

For the antioxidant activity experiments, honey samples were dissolved in 70% methanolic water solution and the solution were diluted to the appropriate concentrations.

For the saccharide analysis, 0.15 g of the honey sample was dissolved in 30 ml of deionized water and stirred for 5 min. For the organic acid analysis, 0.5 g of the honey sample was stirred in 10 ml of deionized water for 10 min. When needed, more concentrated extracts were prepared to increase the sensitivity or the extracts were diluted for the gluconic acid determination.

Determination of total phenolics

The total phenolic contents were determined by the Folin-Ciocalteu procedure [17] using gallic acid as standard. Briefly, 0.1 ml of various concentrations of gallic acid or the methanolic honey solutions (1 mg·ml^{-1}) was diluted with 5.0 ml distilled water. A volume of 0.5 ml of 0.2 g·mEq^{-1} Folin-Ciocalteu reagent was added, and the content was vortexed. Following three-minute incubation, 1.5 ml of Na_2CO_3 (2%) solution was added and, after vortexing, the mixture was incubated for 2 h

at 20 °C with intermittent shaking. The absorbance was measured at 760 nm at the end of the incubation period. The concentration of total phenolic compounds was calculated as gram of gallic acid equivalent per kg of wet honey sample, by using a standard graph.

Determination of the antioxidant activities

The antioxidant activities of the honey samples were determined by two methods, DPPH and FRAP assays. In the first method, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was used to determine antioxidant activities [18]. In the presence of an antioxidant, the purple colour of DPPH decays, and the change of the absorbance can be followed spectrophotometrically at 517 nm. A volume of 1.5 ml of 0.1 mmol·l^{-1} DPPH in methanol was mixed with the equal volume of the methanolic sample solution, shaken well, kept in dark for 50 min, and the activity was measured at 517 nm in the presence of different concentrations of the samples, using BHT, catechin and Trolox as standards. A blank experiment was also carried out to determine the absorbance of DPPH without any sample. SC_{50} (mg·ml^{-1}), the antioxidant concentration to achieve 50% radical scavenging, which was calculated from the curves by plotting absorbance values for corresponding sample concentrations, was used to evaluate the radical scavenging activities of the samples.

The second method is based on the measurement of the iron reducing capacities of honey samples. The working FRAP reagent was prepared by mixing 25 ml of 300 mmol·l^{-1} acetate buffer at pH 3.6 with 2.5 ml of 10 mmol·l^{-1} 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution in 40 mmol·l^{-1} HCl and 2.5 ml of 20 mmol·l^{-1} $FeCl_3.6H_2O$ solution [19]. A volume of 100 μl of the honey sample was mixed with 3 ml of freshly prepared FRAP reagent. Then, the reaction mixture was incubated at 37 °C for 4 min. After that, the absorbance was determined at 593 nm against the blank that was prepared using distilled water and incubated for 1 h instead of 4 min. A calibration curve was used, using an aqueous solution of ferrous sulphate $FeSO_4.7H_2O$ in the range of 100–1 000 μmol·l^{-1} (r^2 = 0.98). In order to make comparison, Trolox was also tested under the same conditions as a standard antioxidant compound. The FRAP values were expressed as millimoles of ferrous equivalent Fe^{2+} per kg of sample.

Determination of individual saccharides

A capillary electrophoretic method developed by our group was used for the analysis of saccharides in the honey samples [20]. The method was based on using a dipeptide, glycylglycine, as the background electrolyte. This electrolyte, without any additive, improved the resolution of saccharides as well as provided their indirect detection. For the honey samples, the optimal separation conditions were selected as 50 mmol·l^{-1} glycylglycine at pH 12.42. The samples were injected at 5 000 Pa for 5 s from the anodic side and the voltage was set at 25 kV. The signal wavelength was set at 350 nm with a reference at 207 nm. Glucose (G) and fructose (F) contents of honey samples were calculated from calibration curves drawn between 2–20 mmol·l^{-1} for both saccharides with 0.994 and 0.997 regression coefficients for glucose and fructose, respectively. *LOD* of the method was 29.2 μg·ml^{-1} and 29.8 μg·ml^{-1} for glucose and fructose, respectively. The precision of the method according to peak areas was 3.08% and 2.83% *RSD* (reproducibility standard deviation) for glucose and fructose, respectively.

Determination of individual organic acids

A capillary electrophoresis method, which was recently applied by us for the analysis of the organic acids in pomegranate juice, was used here for the analysis of organic acids in the honey samples [12]. The analytical method was based on the indirect detection of the organic acids using a chromophore, 2,6-pyridinedicarboxylic acid (PDC), in the separation electrolyte and obtaining fast coelectroosmotic migrations of organic acids by means of dynamic coating of capillary wall with a positively charged surfactant, CTAB. The optimal separation electrolyte was selected as 5 mmol·l^{-1} PDC and 0.1 mmol·l^{-1} CTAB at pH 5.26. The injections were done from cathodic side at 5 000 Pa for 5 s. The running voltage was adjusted to 25 kV. The signal wavelength was set at 350 nm with a reference at 200 nm. The calibration curves were plotted with 5 different concentration levels of the standard samples. The regression coefficients of calibration curves were between 0.997 and 0.999 for all acids.

Statistical analysis

The results were presented as the mean values and the standard deviations (mean ± *SD*). The data were tested using SPSS (version 9.0 for Windows 98, SPSS, Chicago, Illinois, USA). Statistical analysis of the results was based on Kruskal–Wallis test and Pearson correlation analyses. The significant differences were statistically considered at the level of $p < 0.05$ unless otherwise given.

RESULTS AND DISCUSSION

In this study, we investigated in vitro antioxidant activity of methanolic honey samples collected from Black Sea region of Turkey. Chestnut, rhododendron, lime, acacia, cherry, laurel and Anzer honeys are special to Black Sea region. Anzer honey is a heterofloral blossom honey, containing wild flowers from Anzer plateau near Ikizdere and Rize in the East Black Sea Region, Turkey. This honey is the one of the most famous honey types in Turkey and exported to many countries. Pine honey is a special type of honey which is produced by honey bees from the honeydew excreted by an insect *Marchalina hellenica*. This insect lives by sucking the sap of pine trees. This honey is produced only in Turkey and Greece. The plant endemic to the Black Sea rhododendron species, *Rhododendron ponticum*, gives a honey which is locally known as "mad" or wild honey, and is being produced only in the Black Sea region [6, 21]. This honey contains grayanotoxins (formerly known as andromedotoxins, acetylandromedol or rhodotoxins), polyhydroxylated cyclic diterpenes. *Rhododendron ponticum* leaves and flower nectar (including honey made from plant nectar) are sources of these toxins. The symptoms of poisoning due to the consumption of large amounts of this honey include sudden severe vertigo, arterial hypotension and bradycardia [21, 22].

In this study, we compared total phenolic contents and antioxidant activities of the honey samples from different sources with Folin-Ciocalteu assay and DPPH and FRAP methods, which are the common methods preferred by researchers for total phenolic contents and antioxidant activities. The FRAP test is considered to be a good indicator for total antioxidant power because total reducing power is the sum of the reducing powers of individual compounds present in a sample**.** The increased absorbance is an indication of higher reducing power in this method. These methods might suffer from some interference [23, 24]. However, they can be used to compare similar samples. In the study, these assays facilitated comparison of honey samples in Black sea region and to compare data on honeys of the same origin published in the literature.

The total phenolics and the antioxidant activities of the honey samples are given in Tab. 1. As can be seen from the table, the highest values of total phenolics belong to the chestnut honey samples (S1, S2, S4) and the pine honey (S7), following honeys S6 and S10, which are Anzer honeys. The honey richest in total phenolic content was found among chestnut honeys and pine honeys. In these samples, total phenolic contents were found relatively high in darker honey samples as seen from Tab. 1. The highest FRAP values and the lowest values of SC_{50} (mg·ml^{-1}) were obtained for the chestnut honey, pine honey and Anzer honey samples. The reducing power measured for all honey samples showed a concentration dependent pattern. We found a high positive correlation between the phenolic content and FRAP values ($r^2 = 0.86$, $p < 0.05$). Therefore, the correlations

Tab. 1. Antioxidant activities, total phenolics and colours of the honeys from different botanical sources and regions.

Botanical origins and regions	Code	Total phenolics[a] [g·kg^{-1}]	FRAP[b] [mmol·kg^{-1}]	DPPH SC_{50} [mg·ml^{-1}]	Colour[c]
Chestnut (Zonguldak, West Black Sea)	S1	1.13 ± 0.07	338.05 ± 3.04	60.06 ± 5.07	2.980
Chestnut (Eregli, West Black Sea)	S2	1.14 ± 0.02	346.24 ± 4.08	61.41 ± 4.08	2.540
Chestnut (Görele, East Black Sea)	S4	0.95 ± 0.03	235.87 ± 7.25	107.23 ± 11.63	2.260
Rhododendron (Eregli, West Black Sea)	S3	0.63 ± 0.03	117.23 ± 7.15	224.86 ± 12.02	0.580
Rhododendron (Görele, East Black Sea)	S8	0.68 ± 0.02	113.00 ± 3.20	770.05 ± 52.82	0.610
Anzer flowers (Anzer plateau, East Black Sea)	S6	0.90 ± 0.04	244.13 ± 4.46	102.12 ± 11.15	1.440
Anzer flowers (Anzer plateau, East Black Sea)	S10	0.88 ± 0.06	153.02 ± 2.20	91.23 ± 6.01	1.310
Flowers (Gümüşhane, East Black Sea)	S5	0.38 ± 0.02	67.69 ± 4.07	975.12 ± 25.08	0.550
Acacia (Ordu, Middle Black Sea)	S9	0.36 ± 0.02	31.32 ± 3.40	1355.06 ± 74.45	0.390
Pine (Mugla, Aegean)	S7	0.94 ± 0.03	330.12 ± 2.11	70.63 ± 8.71	2.040
BHT [μg·ml^{-1}]		–	2159 ± 12	5.10 ± 0.25	–
Trolox [μg·ml^{-1}]		–	–	9.90 ± 0.15	–

a – total phenolics are expressed as g of gallic acid per 1 kg of honey, b – FRAP values are expressed as mmol of Fe^{2+} per 1 kg honey, c – colour values are expressed as Pfund index of 560 nm absorbance.

Tab. 2. Glucose (G) and fructose (F) contents and F/G ratios of the studied Turkish honeys.

Botanical origins and regions	Code	Fructose [g·kg^{-1}] [a]	Glucose [g·kg^{-1}] [b]	F/G
Chestnut (Zonguldak, West Black Sea)	S1	538.10 ± 8.10	331.50 ± 3.00	1.62
Chestnut (Eregli, West Black Sea)	S2	471.70 ± 3.00	328.20 ± 1.00	1.44
Chestnut (Görele, East Black Sea)	S4	642.20 ± 4.00	367.60 ± 1.80	1.75
Rhododendron (Eregli, West Black Sea)	S3	537.30 ± 3.00	340.20 ± 9.80	1.58
Rhododendron (Görele, East Black Sea)	S8	446.10 ± 21.00	322.00 ± 5.50	1.39
Anzer flowers (Anzer plateau, East Black Sea)	S6	443.20 ± 15.10	374.40 ± 7.20	1.18
Anzer flowers (Anzer plateau, East Black Sea)	S10	532.40 ± 16.10	346.60 ± 5.40	1.54
Flowers (Gümüşhane, East Black Sea)	S5	602.20 ± 2.00	422.40 ± 5.40	1.43
Acacia (Ordu, Middle Black Sea)	S9	567.30 ± 3.00	339.10 ± 4.60	1.67
Pine (Mugla, Aegean)	S7	310.20 ± 2.00	223.50 ± 3.90	1.39

a – values are expressed in g fructose content per 1 kg of honey, b – values are expressed in g glucose content per 1 kg of honey.

obtained between the antioxidant capacities from the two methods and the total phenolic contents suggest that phenolic compounds are mostly responsible for the antioxidant effects of honey. This positive correlation matches the data reported by other researches [7, 8, 25]. There was a negative relationship between the FRAP values and DPPH ($r^2 = –0.66, p < 0.05$), as well as the phenolic content and DPPH ($r^2 = –0.78, p < 0.05$) of the honey samples. The decreased SC_{50} value was an indication of higher radical scavenging activity of DPPH radical in this method. The high antioxidant capacities of the chestnut honey samples were reported by several researches [6, 25, 26].

The colours of the Turkish honeys in this study were very variable and ranged from white to dark amber. With regard to colour measurements based on Pfund scale, chestnut and pine honey samples showed darker colour than the blossom honeys ($p < 0.05$). The brightest honey was acacia, being almost colourless to white. We obtained a positive correlation between total phenolics and colour ($r^2 = 0.87$), and also between the FRAP values and colour ($r^2 = 0.87$). The correlations between the parameters analysed were found to be statistically significant ($p < 0.05$). The positive correlation means that darker honeys have higher antioxidant activity because of their higher contents of total phenolics. The colour of honey is related to the contents of pollen, phenolics, mineral composition, hydroxymethylfurfural and is characteristic of floral origin. Many researchers found that honeys with dark colour have a higher total phenolic content and consequently a higher antioxidant capacity [5, 7, 27]. Because of their high contents of phenolic constituents, they may also possess the biological active properties.

Fig. 1 shows the electrophoregram of one of the honey samples (S4). Glucose (G) and fructose (F) contents and F/G ratios of ten studied honey samples were given in Tab. 2. The main saccharides in all honey samples were found to be glucose and fructose, which is a characteristic of honey. As seen from Tab. 2, glucose contents were between 223.50 g·kg^{-1} and 422.40 g·kg^{-1}, and fructose contents were between 310.20 g·kg^{-1} and 642.20 g·kg^{-1}. The reproducibility of peak areas was 3.08% and 2.83% *RSD* for glucose and fructose, respectively, which is an acceptable value for capillary electrophoresis. The exceeding glucose and fructose contents for samples S4 and S5 were inside the standard deviation of results. Saccharose was not detected in honeys, being either ab-

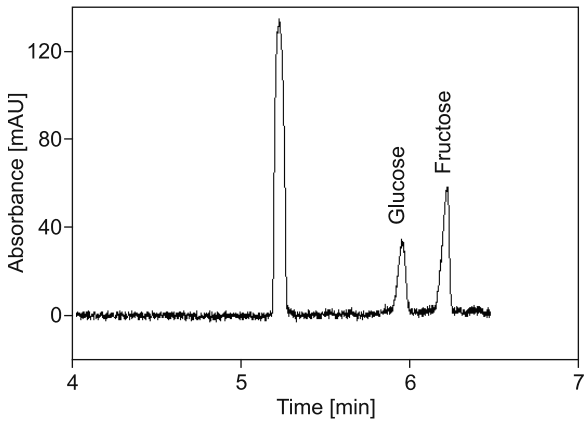

Fig. 1. Electrophoregram of the honey S4.

Conditions: capillary 56.5 cm effective length x 50 μm I.D; separation electrolyte 50 mmol·l^{-1} glycylglycine; pH 12.42; voltage 25 kV.

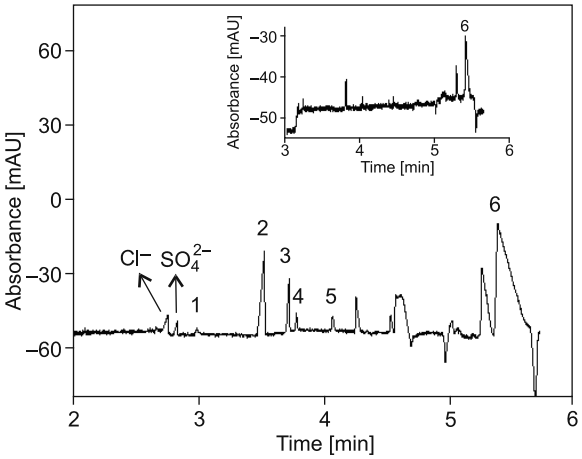

Fig. 2. Electrophoregram of the honey S1.

Conditions: capillary 56.5 cm effective length x 50 μm I.D; separation electrolyte 5 mmol·l^{-1} PDC; 0.1 mmol·l^{-1} CTAB; pH 5.26; voltage –25 kV.
Organic acids are given as: 1 – oxalic acid, 2 – formic acid, 3 – malic acid, 4 – citric acid, 5 – succinic acid, 6 – gluconic acid.
On-set electrophoregram shows the analysis of a diluted honey extract at quantification of gluconic acid.

sent or its contents were below the detection limit of method.

The F/G ratio is specific for fruit juices and is one of the fingerprints for detection of adulteration of fruit juices [12]. Similarly, the sum or ratio of glucose, fructose and water were found to be more specific indicators of the honey quality than any individual parameter [28]. F/G ratio of ten honey samples in this study changed between 1.18 and 1.75 with an average value of 1.50. For comparison, F/G ratios of honeys from different origins were reported to be between 0.97 and 1.86 [26, 27], 1.13 and 1.39 [28, 29], 1.1 and 1.7 [30, 31]. Honey is a highly valuable product. The composition of these products is rather variable and depends on many geographical conditions such as the plant types, climate, environmental conditions and contribution of the beekeeper [3]. However, some of the factors such as overfeeding with saccharose and other saccharides, veterinary drugs and storage conditions affect honey quality and composition.

Tab. 3. Organic acid contents of the studied Turkish honeys.

Botanical origins and regions	Code	Oxalic acid [a] [mg·kg^{-1}]	Formic acid [a] [mg·kg^{-1}]	Malic acid [a] [mg·kg^{-1}]	Citric acid [a] [mg·kg^{-1}]	Succinic acid [a] [mg·kg^{-1}]	Gluconic acid [b] [g·kg^{-1}]
Chestnut (Zonguldak, West Black Sea)	S1	NQ	1276 ± 3	476 ± 13	211 ± 6.0	141 ± 12.0	8.904 ± 0.22
Chestnut (Eregli, West Black Sea)	S2	NQ	940 ± 47	878 ± 5	465 ± 7.0	232 ± 3.4	10.93 ± 0.17
Chestnut (Görele, East Black Sea)	S4	NQ	929 ± 11	105 ± 7	78.9 ± 6.70	39.0 ± 2.5	13.78 ± 0.53
Rhododendron (Eregli, West Black Sea)	S3	ND	4.7 ± 0.6	164 ± 1	NQ	41.7 ± 0.3	6.514 ± 0.35
Rhododendron (Görele, East Black Sea)	S8	ND	40.0 ± 2.9	162 ± 1	NQ	NQ	5.790 ± 0.06
Anzer flowers (Anzer plateau, East Black Sea)	S6	ND	17.3 ± 0.5	ND	ND	ND	5.516 ± 0.09
Anzer flowers (Anzer plateau, East Black Sea)	S10	ND	ND	ND	ND	ND	4.223 ± 0.02
Flowers (Gümüşhane, East Black Sea)	S5	ND	ND	ND	ND	ND	2.012 ± 0.06
Acacia (Ordu, Middle Black Sea)	S9	ND	ND	ND	ND	ND	1.501 ± 0.05
Pine (Mugla, Aegean)	S7	NQ	89.3 ± 5.9	257 ± 32	124 ± 15.0	NQ	3.467 ± 0.30

ND – not detectable, NQ – not quantifiable.
a – values are expressed in mg oxalic acid, mg formic acid, mg malic acid, mg citric acid and mg succinic acid per 1 kg of honey,
b – values are expressed in g gluconic acid per 1 kg of honey.

Fig. 2 shows the electrophoregram of one of the honey samples (S1) for organic acid analysis. On-set electrophoregram shows the diluted honey extract in order to determine the quantities of gluconic acid content. The organic acid contents of honey samples were given in Tab. 3. There is a limited amount of literature data on the individual organic acid contents of honey samples. Gluconic acid contents found in honeys from 10 different botanical origins were between 8 $g \cdot kg^{-1}$ and 12.3 $g \cdot kg^{-1}$ [15]. It has been reported that the amount of gluconic acid in 48 samples from 4 different botanical origins were 2–11.6 $g \cdot kg^{-1}$ [32]. Gluconic acid contents of 57 samples from 9 different botanical origins were reported to be between 1.766 $g \cdot kg^{-1}$ and 4.933 $g \cdot kg^{-1}$ [32] and for broom honeys as 13.5–26.1 $g \cdot kg^{-1}$ [30].

Malic, citric and succinic acid contents were found also in accordance with literature findings for honeys of different botanical origins [15, 33]. These acids were either absent in honeys or present only in small quantities depending on the honey origin. The formic acid contents for S1, S2, and S4 (Chestnut honeys) are higher than those of 46–908 $mg \cdot kg^{-1}$ reported by MATO et al. [15], and 50–506 $mg \cdot kg^{-1}$ reported by SUAREZ-LUQUE et al. [14]. Formic acid is a natural honey acid but it is also used against bee mites, as there currently exists no restriction on the levels and no report on risks to human health.

Organic acids in honey samples might contribute to the antioxidant capacity of honeys either by their own antioxidant properties or by enhancing the effect of other antioxidant compounds by changing the acidity of honeys. We studied the correlation between the gluconic acid contents and the antioxidant values of the honeys. The correlation coefficient between gluconic acid levels versus FRAP values and DPPH values were found as 0.57 and 0.51, respectively.

As a conclusion, we reported here the total phenolic contents and the antioxidant activities of ten authentic Turkish honey samples from the Black Sea region of Turkey, together with their individual organic acid and saccharide contents. All the honey samples showed good antioxidant activities, correlating to their total phenolic contents. Chestnut honeys, pine honey and Anzer honeys showed higher phenol contents and antioxidant activity values, promising to be a good source of natural health-beneficial antioxidants for the diet. The types and the levels of the organic acids varied in the honey samples, which are mainly responsible for the different flavour of the honey samples. Fructose and glucose ratio, which is an indication of saccharide adulteration, was found within the acceptable values. For a fast and a simple analysis of organic acids and saccharides in the honey samples, the capillary electrophoresis method is recommended.

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