MELLIFERA RESEARCH ARTICLE

Bioactive Properties of Blossom and Honeydew Honeys

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A B S T R A C T

In this study, it was aimed to determine the bioactive properties of honeydew and blossom honeys produced in Turkey. Botanical origins of honey samples (locust, sunflower, citrus, lavender, coriander, euphorbia, rhododendron, chestnut, carob, thyme, rape, linden, pumpkin, heather, nigella, milk thistle, pine and oak honeys) collected from different geographical regions have been dertemined by pollen analysis. Total phenolic content of honey samples were determined by Folin-Ciocalteu method. The total phenolic content belongs to rape honey with the lowest 70.60 mgGAE/100g and chestnut honey with the highest 212.06 mgGAE/100g. Antioxidant activity of honey samples was determined by phosphomolybdenum method and antiradical activity by DPPH method. The lowest antioxidant activity was found in lavender honey and the highest activity was in citrus honey. The highest antiradical activity was determined in chestnut honey with the lowest antiradical activity in thyme honey; 66.02% and 7.47%, respectively.

Keywords: blossom honey, honeydew honey, phenolic content, antioxidant activity

Introduction

Honey is a sweet and natural food where plant nectar or some insect secretions are collected and processed by honey bee (*Apis mellifera* L.) and stored in the honeycomb cell. According to the source of honey is divided into two classes as honeydew and blossom honeys. Blossom honey is produced by honeybees from the nectar of plants. Honeydew honey is formed by collecting and processing digestive residues of basra (*Marchelina hellenica*) insects fed from the sap of plants [1]. Honeys such as citrus, chestnut, heather and thyme are among the blossom honeys; pine and oak honeys are among the honeydew honeys.

The botanical origin of honey has been determined by pollen analysis, a method that is called melissopalynolgy. The method of pollen analysis, which was elaborated and proposed by the International Commission for Bee Botany

(ICBB) in 1970, and updated in 1978 [2]. In addition, EU Council Directive (2002) related to honey, it is indicated that the product names may be supplemented by information referring to floral origin, if the product comes mainly or wholly from the indicated source and possesses microscopic, organoleptic and phsico-chemical characteristics of source. The determination of the botanical origin is based on the relative frequencies of nectariferous taxon's pollen types. The frequency classes of pollen grains were given as predominant (>45%), secondary pollen (15-45%), important minör pollen (3-15%) and minör pollen (1-3%). Honey can be defined as unifloral if the "characteristic" pollen exceeds 45%. In addition, it is considered honeydew honey if the ratio HE/PG" exceeds 3. Howeever many pollen types are underrepresented (*Citrus* spp., *Tilia* spp., *Robinia pseudoacacia*) or over-represented (*Eucalyptus* spp., *Castanae sativa*). For instance, to characterize citrus honey as unifloral, *Citrus* spp. pollen must be over 10% while, for chestnut honey, a content of 90% of *Castanea sativa* pollen is required to classidy honey as unifloral [3]. However, in polifloral/multfiloral honeys, no dominant pollen is contained. Therefore, it is generally named according to the geographic region from which multifloral honey is obtained [4-6]. The total amount and composition of phenolic compounds in honey varies depending on the plant species in which the bee collects nectar, the method of collecting the nectar, seasonal and environmental factors, geographical origin and storage conditions. Total phenolic content in honey varies between 5 and 1300 mg/kg. It is strongly believed that the source of phenolic substances in honey is propolis. The flavonoid content, which is an important group of phenolic substances, is approximately 0.5% in pollen, 10% in propolis and about 0.005-0.01% in honey [7].

It is reported that the total phenolic content of honey is related to antioxidant activity Therefore, abundant phenolic compounds are found in dark honeys and such honeys are reported to be more powerful antioxidants than ascorbic acid or vitamin E [8,9]. According to Nagai et al., vitamins B1, B2 and C are degraded in heat treated honeys, and antioxidant activity decreases rapidly as a result of the destruction of peroxidase and catalase enzymes [10].

It is known that dark honeys have higher phenolic content and have higher antioxidant activity than light ones. In a previous study, the phenolic acid and flavonoid contents of blossom and

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honeydew honeys produced in different geographical regions of Turkey were determined and chestnut honey has been reported to contain the highest phenolic content (0.05 mg/g GAE) [9]. In another study, Perez et al. reported that Spain's honeydew honeys have higher antioxidant activity than blossom honeys [11].

Turkey, in terms of honey production and diversity is the country with the best potential in the world. Unfortunately, studies on the biological activity of monofloral honey produced in Turkey is not enough. Therefore, in this study, it was aimed to determine the bioactive properties of 48 different honeydew and blossom honeys produced in Turkey.

Materials and Methods

Honey samples

Honey samples from different provinces of Turkey (Istanbul, Edirne, Antalya, Isparta, Zonguldak, Izmir, Bursa, Mersin, Artvin, Şanlıurfa) were obtained from beekerpers in 2016 (Table 1). In the study,18 different monofloral honeys (locust, sunflower, citrus, lavender, coriander, euphorbia, rhododendron, chestnut, carob, thyme, rape, linden, pumpkin, heather, nigella, milk thistle, pine and oak) were stored in a dark and cool conditions (+4 °C) until analyzed. A total of 48 honey samples were analyzed. All analyzes of the samples were carried out during the year the honeys were produced.

Pollen analysis of honey samples

Pollen analysis of honey samples has been recognized by international beekeeping authorities, Louveaux et al. [2]. Briefly, honey samples were kept in a 45 °C water bath for 10-15 minutes and homogeneity was achieved by mixing. Then 5 g of honey and 10 g of distilled water were mixed in falcon tubes, the mixture was vortexed and centrifuged at 6500 rpm for 20 minutes. Then, water in centrifuged tubes was removed and tubes were left upside down for full drainage. The sediment material was taken from the bottom of the tube and plated on a lam with glycerin gelatin mixture. Glycerin-gelatine mixture and honey were taken with the edge of a sterile needle was transferred to a microscope slide and put on a hotplate set at 40°C. When the gelatine was melted, 18×18 mm cover slips were placed on the samples. Pollen slides were researched with Nickon E 200 microscope and immersion objective (x100) was used for identification of pollens. During microscopic studies all the area, which is 18x18 mm, was checked, 200 pollen was counted for each sample. [12].

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Determination of total phenolic content

Total phenolic content in honey was determined by Folin-Ciocalteu method and read spectrophotometrically [13]. Briefly,1 g of honey sample was made up with 4 mL (1: 4) methanol and vortexed. The prepared solution was filtered through Whatman No. 1 paper. The stock concentrations of the samples were prepared to be 200,000 ppm. Sample incubated at room temperature and dark for 2 hours. The absorbance of the resulting mixtures was read on the spectrometer against the blank at 765 nm wavelength. The spectrophotometer values of the samples were converted according to the formula prepared using the regression coefficient of gallic acid. The total phenolic content of the samples was expressed as mg gallic acid equivalent (GAE/100 g honey) [14].

Determination of antioxidant activity

Antioxidant activities of honey samples were determined according to the phosphomolybdenum method [15]. One g of honey sample was vortexed by adding 9 mL of methanol. The prepared solutions were allowed to stand in a 95 ^{o}C water bath for 90 minutes then cooled in tap water. The absorbance of the samples was read on the spectrophotometer at 695 nm wavelength. Antioxidant activity values of honey samples were expressed as mg ascorbic acid equivalent (AAE/g honey) [14].

Determination of antiradical activity

Free radical scavenging activities of the samples were determined by DPPH (2,2 diphenyl-1-picrylhydrazyl) method by making some modifications in the analysis protocols [16]. One g of honey sample and 4 mL of methanol were vortexed with stirring. 100 µL of this solution was added and 3900 µL of DPPH (1000 µl of 6 × 10^{-5} M DPPH) prepared in methanol was added and the mixture was allowed to stand at room temperature and in the dark for 2 hours. Their absorbance was read on the spectrophotometer at a wavelength of 517 nm.

Statistical Analysis

All chemical assays were carried out in triplicate and the data were expressed as means ± standard deviations (SD).

Results and Discussion

As a result of pollen analysis, it was found that some of the honeys labeled according to beekeeper claims were not monofloral honey. For instance, thyme and carob honeys. Locust, sunflower,linden, lavender, citrus and rhododendron honeys do not contain more than> 45% pollen but they are defined as “unifloral” honey because they show under-represented pollen properties. Other blossom honeys tested were

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identified as “multfiloral”. Pine and oak honey has a honeydew honey feature because the HE/PG value is 3 (Table 1).

Table 1. Botanical and geographical origin, pollen frequency of honey samples

Honey	**n**	**Botanical origin**	**Geographical origin**	**Pollen frequency (%)**
Locust	3	*Robinia pseudoacacia* L.	Muğla	39.70
Sunflower	3	*Helianthus annus* L.	Edirne	38.85
Pine	4	*Pinus spp.*	Muğla	HDE/P>3*
Nigella	2	*Nigella sativa* L..	Antalya	37.38
Heather	2	*Vitex agnus-castus*	Isparta	40.6
Linden	3	*Tilia platyphyllos Scop.*	Zonguldak	41.43
Pumpkin	3	*Cucurbita pepo* L.	Antalya	40.25
Rape	2	*Brassica napus* L.	Diyarbakır	41.77
Carob	3	*Ceratonia siliqua* L.	Antalya	39.15
Thyme	3	*Thymus vulgaris*	Isparta	36.87
Chestnut	4	*Castanea sativa Miller*	Bursa	92.10
Coriander	2	*Coriandrum sativum* L.	Antalya	40.97
Lavender	3	*Lavandula stoechas* L.	Isparta	41.85
Oak	2	*Quercus robur* L.	Zonguldak	HDE/P>3*
Citrus	2	*Citrus spp.*	Mersin	38.92
Rhododendron	3	*Rhododendron* L.	Artvin	35.21
Euphorbia	2	*Euphorbia macroclada Boiss.*	Şanlıurfa	40.72
Milk thistle	2	*Slybum marianum* L.	Diyarbakır	41.45

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Table 2. Total phenolic content, antioxidant and antiradical activity of honeys (Mean±SD)

Honey	Total Phenolic Content (mg GAE/100 g honey)	Antioksidant Activity (mg AAE/g honey)	Antiradical Activity (% inhibition)
Locust	103.45±3.37^{bc*}	83.78±1.71^{g*}	17.39±1.38^{bc}
Sunflower	110.17±4.70^{bc}	98.88±1.44^{h}	41.83±1.90^{de}
Pine	192.30±18.03^{f}	63.42±7.81^{cd}	40.05±22.06^{de}
Nigella	190.13±5.34^{f}	82.40±1.76^{fg}	11.00±0.64^{ab}
Heather	106.47±5.28^{bc}	129.57±11.63^{i}	10.33±0.31^{ab}
Linden	116.90±10.00^{c}	67.61±12.18^{de}	14.19±2.39^{abc}
Pumpkin	75.60±2.51^{a}	45.80±5.56^{ab}	17.47±1.76^{bc}
Rape	70.60±8.01^{a}	70.93±5.40^{def}	46.88±1.96^{e}
Carob	153.40±6.71^{de}	74.41±3.40^{defg}	20.94±1.21^{c}
Thyme	158.25±13.96^{de}	77.07±1.11^{efg}	7.47±1.29^{a}
Chestnut	212.06±12.41^{g}	79.57±2.20^{fg}	66.02±0.97^{f}
Coriander	118.92±6.58^{c}	135.26±1.77^{i}	44.09±0.12^{de}
Lavender	145.12±7.30^{d}	38.30±7.14^{a}	44.83±0.37^{f}
Oak	209.00±32.66^{g}	56.36±21.09^{bc}	41.63±1.29^{de}
Citrus	97.47±2.58^{b}	138.28±2.71^{i}	12.64±1.47^{abc}
Rhododendron	165.35±9.55^{e}	48.70±3.15^{ab}	7.61±1.06^{a}
Euphorbia	153.04±2.83^{de}	84.12±2.03^{g}	11.43±0.78^{ab}
Milk thistle	108.73±3.56^{bc}	103.42±1.99^{h}	43.69±0.28^{de}

Different letters in the same column represent statistically different groups (p <0.05).

As a result of the analysis, a statistically significant difference was found between the total phenolic contents of honey (p <0.05). The total phenolic content of the honeys tested ranged from 70.60-212.06 mg GAE/100 g honey. The total amount of phenolic substances belongs to rapeseed honey with the lowest 70.60 mg GAE/100

g honey and the highest amount of chestnut honey with 212.06 mg GAE/100 g honey. The highest total phenolic content after chestnut honey belongs to oak and pine honey.

The difference between antioxidant activity of honey was found to be statistically significant ($p < 0.05$). Among the honeys tested, the highest antioxidant activity was detected in citrus, coriander and heather honeys, 138.28,135.26 and 129.57 mgAAE/g honey, respectively. The lowest antioxidant activity was found in lavender honey.

The antiradical activity of the honeys analyzed varied between 7.47-66.02%. The highest antiradical activity was found in chestnut honey and the lowest activity was found in thyme honey.

According to the results of this study, labeling honeys according to beekeeper claims can be misleading. Melissopalinological determination of botanical origin of honey is based on the relative frequency of the pollen from the nectar-secreting plant. It is known that this method is time-consuming, requires knowledge and expertise, and involves a laborious counting procedure. In adidition, some difficulties in this method are associated with the need of good experince and knowledge of pollen morphology and the availability of collection of pollen [17]. However, some studies on the chemical composition of honey were performed without pollen analysis, in such cases, botanical origin of honey was based on the claims of local beekepers, when determination of honey origin is performed by considering the predominant flowers surrounding the hive. Even though pollen analysis has some disadvantages or limitations, it is the only way to detect contribution of nectar from other floral origin.

It has been reported in some studies that dark honeys are rich with phenolic compounds and antioxidant activities of honeys with high phenolic content are high. [18,19]. Lachman et al. determined the total phenolic content and antioxidant activity in 40 Czech honeys. The researchers found that the total phenolic content of honey varies between 83.60-242.52 mg GAE/kg [20]. In our study, the total phenolic content of honeys was between 70.59-212.06 mg GAE/100g honey. As a result of the analysis, the highest phenolic content was found in chestnut and honeydew honeys and the lowest in rape honey. In a study by Haroun, it was reported that the total phenolic content of chestnut honey varies between 33.37-77.40 mg GAE/100 g honey [9]. In our study, it was found that the

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phenolic content of the chestnut honey was higher than the values reported by the researcher. Akbulut et al. examined the antioxidant activity and phenolic content of 15 pine honey samples from different regions of Muğla. According to the total phenolic analysis, they found that the polyphenol content of honeys was in the range of 234.9-394.0 mg/100 g [21].

Many researchers reported that there was a significant relationship between antioxidant activity and total polyphenol content of honey. In a study by Silici and Özkök analyzed 66 honey bee products (honey, pollen, royal jelly, propolis) and their mixtures. They determined the total phenolic content of honey samples between 57.59-261.71 mg GAE/100g. Researchers reported that the total phenolic content of honeys examined in citrus honey with the lowest value of 57.59 mg GAE/100 g, and the highest 261.71 mg GAE/100 g for chestnut honey [22]. According to the findings of our study, total phenolic content of citrus honey is higher than (97.47 mg GAE/100 g) their value (57.59 mg GAE/100 g). Although total phenolic content of chestnut honey 212.06 mg GAE/100 g was lower than the value found by Silici and Özkök [22]. In another study, Al et al. was found that, the total amount of phenolic substances were in sunflower 45.00, in linden 38.00 and in multifloral honey 23.00 mg GAE/100 g. In another study, total phenolic content was found to be 110.17 in sunflower and 116.89 GAE/100 g in linden honeys [23]. Therefore, it can be said that the total phenolic content may vary depending on plant origin, climatic conditions and environmental factors from which honeys are taken.

It is known that, the main sources of honey phenolic compounds are plants. Plants biosynthesize a great number of phytochemicals and antioxidants being the major group of bioactive constituents, which might reduce the risk of oxidative damage in living cells [24]. It is shown that honey, depending on the floral source, possesses higher or lower antioxidant or antiradical activity [25,26]. The composition of phytochemicals has an influence on the biological activity of honey; usually, the same compounds have antioxidant activity. Many studies reported that the composition of honey depends on the floral source used to collect nectar; however, seasonal and environmental factors, as well as processing, may also have an effect on the composition of phenolic compounds in honey [26-28].

In honey, phenolic compounds are among the components responsible for antioxidant

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properties [29]. The highest antioxidant activity in the honeys analyzed was found to be citrus 138.28 mg AAE/g and the lowest lavender honey was 38.30 mg AAE/g. Among the honey samples tested, honeys with the highest antioxidant activity were citrus, coriander and heather honey, 138.28, 135.26 and 129.57 mg of AAE/g, respectively. Honeys with the lowest antioxidant activity were lavender 38.30 and pumpkin 45.80 mg AAE/g. Buratti et al. reported that the differences between honey antioxidant activities of different geographical origin of honeys may be due to such as environmental and climatic conditions, ie temperature, humidity, soil structure [30]. Antiradical activity of honey was found to be 7.47% in thyme honey and 66.02% inhibition in chestnut honey (Table 2). There was a statistically significant difference between honeys in terms of total antiradical activities ($p < 0.05$). Akbulut et al. collected pine honeys from Muğla and found antiradical activity of honeys were 35.32%. However, they reported a high correlation between antiradical activity and phenolic content ($r = 0.887$) [31]. They emphasized the importance of pine honey as a good antioxidant source. Our results are similar to the findings of the researchers (40.05% inhibition) in terms of antiradical activity.

Conclusion

It may be concluded that the presence of many factors, which might have an effect on the total phenolic content and antioxidant activity of honey as well as structural variety of such constituents that are biosynthesized by the floral sources of honey.Turkey has a very rich flora makes it possible to produce a large number of different monofloral honey. Pollen and bioactive properties of honey samples obtained in this study were determined and in this sense, contribution was made to the literature. In the following studies, the investigation of other honey types that cannot be included in this study will be complementary to the deficiency in this subject.

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Çiçek ve Salgı Ballarının Biyoaktif Özellikleri

Öz: Bu çalışmada, Türkiye'de üretilen çiçek ve salgı ballarının biyoaktif özelliklerinin belirlenmesi amaçlanmıştır. Farklı coğrafik bölgelerden toplanan bal örneklerinin (akasya, ayçiçek, narenciye, lavanta, kişniş, sütleğen, ormangülü, kestane, keçiboynuzu, kekik, kolza, ıhlamur, kabak, hayıt, çörekotu, devedikeni, çam, meşe) polen analizi yapılarak botanik orijinleri tespit edilmiştir. Balların toplam fenolik madde içeriği Folin-Ciocalteu metodu ile belirlenmiştir. Toplam fenolik madde miktarı en düşük 70.60 mg GAE/100 g bal ile kolza balına ve en yüksek 212.06 mg GAE/100 g bal ile kestane balına aittir. Bal örneklerinin antioksidan aktivitesi fosfomolibden metodu, antiradikal aktivitesi ise DPPH metodu ile belirlenmiştir. En düşük antioksidan aktivite lavanta balında en yüksek aktivite ise narenciye balında belirlenmiştir. Analiz edilen ballarda en yüksek antiradikal aktivite% 66.02 ile kestane balında, en düşük antiradikal aktivite %7.47 ile kekik balında belirlenmiştir.

Anahtar Kelimeler: çiçek balı, salgı balı, fenolik içerik, antioksidant aktivite

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