



## Characterization of Anatolian honeys based on minerals, bioactive components and principal component analysis



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### ABSTRACT

Our aim is the characterization of Anatolian monofloral and honeydew honeys according to their mineral, vitamin B2, total phenolic contents and antioxidant activities. Five main elements (Ca, K, Fe, Cu, and Mn) were determined in 20 honey samples by inductively coupled plasma – optical emission spectrometry (ICP-OES). The vitamin B2 contents of honey samples were determined by the capillary electrophoresis method coupled with a sensitive laser induced fluorescence (LIF) detector. The total phenolic contents were analyzed with Folin–Ciocalteu's method. Two comparative antioxidant assays, namely cupric reducing antioxidant capacity assay (CUPRAC) and ABTS radical scavenging assay, were applied to detect the antioxidant power of honeys. Heather honeys were distinguished from others with significantly high vitamin B2 and iron contents. Considerably higher antioxidant capacities and Mn contents were observed for oak and chestnut honeys. Principal components analysis was applied to the analysis result in order to classify the honeys from different botanical origins.

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### 1. Introduction

Honey is a natural product which is well known for its valuable nutritional and medicinal qualities. The composition of the healthy ingredients of honey, except for its sugars, is strongly associated with its floral origin. In recent years, there has been an increasing demand to monofloral honeys in markets. Monofloral honeys are more expensive than multi-floral ones. Such foods with high commercial value are targets for adulteration. With food adulteration, not only consumers are being defrauded, but also economies of regions and countries are adversely affected due to unfair competition. Therefore, it is important to display the important ingredients of monofloral honeys consumed worldwide. Many

reports displaying the ingredients of honeys from several countries have been published. They are mainly on mineral contents (Akbulut, Ozcan, & Coklar, 2009; Bağcı, Arslan, Ozcan, & Dursun, 2007; Kolaylı et al., 2008; Küçük et al., 2007; Madejczyk & Baralkiewicz, 2008; Özcan, Ölmez, Arslan, & Dursun, 2012; Vanhanen, Emmertz, & Savage, 2011) and antioxidant activities together with total phenolics of honeys (Alvarez-Suarez et al., 2012; Küçük et al., 2007). There are also reports on specific phenolics (Can et al., 2015; Marshall, Schneider, Cisneros, & Gu, 2014; Perna, Intaglietta, Simonetti, & Gambacorta, 2013), organic acids (Daniele, Maitre, & Casabianca, 2012; Tezcan, Kolaylı, Sahin, Ulusoy, & Erim, 2011), amino acids (Kivrak, 2014; Silici & Karaman, 2014) and vitamin contents (Ciulu et al., 2011; León-Ruiz, Vera, González-Porto, & San Andrés, 2012; Tuberoso et al., 2012; Viñas, Balsalobre, López-Erroz, & Hernández-Córdoba, 2004) of honeys from all over the world. Recently, characterization of different honey varieties with chemometric techniques based on their constituents has been reported. Several authors have applied chemometrical procedures on mineral composition (de Alda-Garcilope, Gallego-Picó, Bravo-Yagüe, Garcinuño-Martínez, & Fernández-Hernando, 2012;

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Fernández-Torres et al., 2005; González-Miret, Terrab, Hernanz, Fernández-Recamales, & Heredia, 2005; Grembecka & Szefer, 2013; Madejczyk & Baralkiewicz, 2008) and mineral composition together with antioxidant activities (Alvarez-Suarez et al., 2012), in order to classify honeys in view of their botanical and geographical origin.

There are many varieties of honeys in Turkey due to the extensive varieties of plants in Anatolia. The main purpose of this work is to evaluate the main mineral composition, antioxidant activity, total phenolic and vitamin B2 contents of 14 monofloral honeys and 6 honeydew honey samples from different botanical origins and different regions of Anatolia. Moreover, principal components analysis were applied to the all data belonging to mineral contents, antioxidant capacities, total phenolics together with vitamin B2 contents for the first time for the possible differentiation of monofloral honeys from different botanical origins.

## 2. Materials and methods

### 2.1. Chemicals

Riboflavin (Vitamin B2), neocuproine, CuCl<sub>2</sub>, and ammonium acetate were purchased from Sigma Chemical Co (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and Folin-Ciocalteu's reagent are from Fluka Chemie GmbH (Buchs, Switzerland). Methanol, acetic acid, hydrochloric acid were obtained from Merck (Merck KGaA, Darmstadt, Germany). All other reagents were of analytical grades.

### 2.2. Honey samples

Seven varieties of unifloral honey, heather, oak, chestnut, pine, astragalus, acacia, and lavender honeys were collected from local experienced beekeepers with the aid of the Turkish Beekeepers Union in 2013 harvest season. Honey types commonly produced in Turkey were planned as samples. Monofloral honeys with highest palynological scores were selected in the study. The abbreviations used for honey samples and their regions are given in Table 1.

### 2.3. Identification of the honey floral

The honey samples were classified as melissopalynological characterization according to their specific botanical variety in the honey samples. The percentage of pollen from the samples was found between 45% and 90%. When a specific type of pollen is over 45%, the honey is referred as monofloral honey (Yao, Jiang, Singanusong, Datta, & Raymont, 2004). Honeydew samples were characterized by optical rotation which were found dextrorotatory in pine and oak honeys. Except pine and oak honeys, all of the samples were exhibited levorotatory properties, and they were called as blossom honeys (Can et al. 2015).

### 2.4. Preparation of honey extracts for analysis

For vitamin B2 analysis, around 1 g of honey was mixed with 2 mL of water under magnetic stirring for 15 min. After this, the extract was filtered with 0.45 µm micro filters and injected directly to the capillary column.

For antioxidant and total phenolic analysis, 10 g sample of honey was placed in a falcon tube and 50 mL methanol was added. The mixture was continuously shaken with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for 24 h and the formed extract was filtered using a Whatman filter paper in order to remove particles. Then the extract was concentrated in a rotary evaporator (IKA-Werke, Staufen-Germany) under reduced pressure at 40 °C. The residue was dissolved in methanol and used for antioxidant tests.

For mineral analysis, 0.5 g of honey was weighed in vessel tubes with 5 mL of concentrated HNO<sub>3</sub> and 2 mL H<sub>2</sub>O<sub>2</sub> (30%). The tubes were incubated 12 h and placed in a microwave oven. Honeys were digested by a Milestone model Ethos D closed vessel microwave system (Milestone, Sorisole (BG)-Italy). During all of the tests, the pressure was kept at 45 bars, and the ventilation was 3 min. Samples were allowed to cool, dissolved in 1 mL concentrated HNO<sub>3</sub>, then made up to 25 mL with distilled water and measured by inductively coupled plasma – optical emission spectrometry (ICP-OES).

All analyses were performed at least three times.

### 2.5. Determination of vitamin B2 contents of honey samples

Vitamin B2 (riboflavin) analysis was performed with an Agilent 1600 capillary electrophoresis system (Waldbronn, Germany) equipped with a ZETALIF 2000 LIF detector (Picometrics, Montlaur, France). Riboflavin was detected with LIF detector at excitation at 488 nm and emission at 520 nm by an Ar-ion laser. The data processing was carried out with the Agilent ChemStation software. The temperature was set at 25 °C. Injections were made at 50 mbar for 6 s. The fused-silica capillary used for separation experiments was 50 µm in diameter and was obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 67 cm and the length to the detector was 50 cm. The new fused-silica capillary was conditioned prior to use by rinsing with 1 M NaOH for 30 min and with water for 10 min. The capillary was flushed successively by 0.1 M NaOH for 2 min, water for 2 min, and buffer for 5 min at the beginning of every working day and between runs.

10 mM Borat at pH 9.6 was selected as the optimal separation medium. The applied voltage was 30 kV and  $\lambda_{\text{ext/em}}$  was 488/520 nm. At these conditions, riboflavin peak migrates in 3.5 min.

The linear calibration range was 0.01–5 µmol/L with a R<sup>2</sup> of 0.999. Limit of detection (LOD), limit of quantification (LOQ), relative standard deviation (RSD %) for intraday (n = 5) and interday (n = 5 × 3) reproducibility values were found as 1.08 ng/mL, 3.58 ng/L, 2.48% and 4.58%, respectively. The recovery values were between 99.8 and 106.0%.

**Table 1**  
Classification of the studied honey samples.

Honey samples	Abbreviation	Local name	Predominant pollen	Collected area of Turkey
Heather	H	Püren	<i>Calluna vulgaris</i>	Muğla
Oak	O	Meşe	<i>Quercus robur</i> L.	Kırklareli
Chestnut	C	Kestane	<i>Castanea sativa</i> Mill.	Trabzon
Pine	P	Çam	<i>Pinus</i> L.	Muğla
Astragalus	As	Geven	<i>Astragalus microcephalus</i> Willd.	Bayburt
Acacia	Ac	Akasya	<i>Robinia</i> L.	Ordu
Lavender	L	Lavanta	<i>Lavandula stoechas</i> L.	Isparta

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