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# Analysis of a Rare Honey Sample From Tuzluca/Iğdır Region

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**ABSTRACT:** Turkey has a wide variety of honey products and most of the honeys are endemic. There are many endemic honey samples in Anatolia that have not been investigated yet. Since natural honey has special benefits due to its many bioactive ingredients, it is still a challenge to classify and characterize different honey samples. In this study, an endemic and rare honey sample from the mountainous and almost uninhabited region of Tuzluca, Iğdır is reported. Honey sample is characterized by the means of antioxidant and antidiabetic capacities, nitrite and nitrate content, fructose/glucose ratio. Results indicate that the reported honey sample has unique characteristics.

**Keywords:** honey, capillary electrophoresis, antioxidant, antidiabetic, Iğdır

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## INTRODUCTION

Honey is known as a valuable nutritional and medicinal food source of natural origin. Its composition is associated with the floral origins, geography as well as presence of pollutants. Turkey is a home to more than 12.000 native plant species and of nearly 450 species are known as honey plants (Sorkun 2008, Özkök et al. 2016). Turkey is second largest honey producer and Anatolia has one of the richest flora (Kaygusuz et al. 2016). In recent years there is an interest to study the characteristics of Turkish honeys and pollen samples. Recent studies include biochemical analysis of Mutki/Bitlis with a good total phenolic concentrations (Özşahin Kireççi and Kireççi 2018), chestnut honeys from Black Sea region with a high fructose+glucose content (Dağ et al. 2017), dandelion (Taraxacum) honey from Bingöl as a new record in Turkey (Özenirler et al. 2018), characterization multifloral honeys of 6 different Sinop districts (Özler 2015), microbiological and parasitological analysis of honey samples from Istanbul (Dümen et al. 2013), volatile compound determination in pine honeys from Muğla-Marmaris region (Silici 2011), characterization of multifloral honeys from Pervari/Siirt (Erez et al. 2015), physicochemical analysis of multifloral samples from Konya and Karaman (Özler et al. 2019), 23 different monofloral honeys across Turkey including Antalya, Ordu, Van, Mardin, Adana, Istanbul, Batman, Konya Izmir, Hatay and Muğla (Gül and Pehlivan 2018), and monofloral honeys from Muğla, Kırklareli, Trabzon, Bayburt, Ordu and Isparta (Kaygusuz et al. 2016), an interesting study reports bio-monitoring of pollutants in industrial districts of Izmir (Aliağa) by using honey bees and propolis (Matin et al. 2016), properties of Jerusalem thorn honey was investigated for Bursa, Edirne and Kırklareli (Malkoç et al. 2019) as well as honey from Aydın (Ünübol Aypak et al. 2019). Such studies are expected to increase in the upcoming years since Anatolian honey sources are numerous and needs to be studied for further discoveries and analyses. Recently honey is reported as an antidiabetic agent (Erejuwa et al. 2012) and it is already known to have good antioxidant characteristics.

In the present paper, properties of a rare honey sample from a mountainous and sparsely populated village of Tuzluca, Iğdır are reported and discussed. The region has an elevation of ~2200 m above sea level. Antioxidant and antidiabetic properties of the sample was investigated as well as some quality parameters such as fructose to glucose ratio and nitrate/nitrite content.

## MATERIALS AND METHODS

### Materials

Honey samples were obtained from Ombulak village in Tuzluca (Iğdır, Turkey). Honey samples were of multifloral origin and were harvested in July 2019 season. α-glucosidase, p-nitrophenyl-α-D-glucopyranoside substrate, D(+) glucose, D(+) fructose and genistein were from from Sigma Chemical Co. (Steinheim, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium nitrite, potassium nitrate, formic acid, sodium sulfate, sodium hydroxide, sodium carbonate, sodium dihydrogen phosphate dihydrate and disodium hydrogen phosphate dodecahydrate were from Merck (Darmstadt, Germany). Glycylglycine were from Fluka (Buchs, Switzerland) and acetonitrile was obtained from J. T. Baker (Deventer, Netherlands). All reagents were used without any further purification

### Capillary electrophoresis

Nitrite-nitrate and sugar analyses were conducted using an Agilent 1600 capillary electrophoresis system (Waldbronn, Germany) equipped with a diode-array detector. Uncoated fused silica capillaries with 50 μm internal diameter (Polymicro Technology, Phoenix, AZ, USA) were used with the total length of 65 cm and active length of 50 cm.

In order to prepare the honey samples for capillary electrophoresis, 0.1 g of honey was mixed with 10 mL of deionized water and stirred at 300 rpm for 30 minutes at room conditions. After mixing completely, samples were filtered using microfilters of 0.45 μm pore size. The resulting filtrate was directly injected for nitrite and nitrate analysis. On the other hand, for sugar analysis the filtrate was diluted two times.

A capillary zone electrophoresis method developed for simultaneous determination of nitrate and nitrite in food samples was employed (Kalaycıoğlu and Erim 2016). Pre-optimized conditions in this method were as follows: 30 mmol/L formic acid solution at pH 4.0 with 30 mmol/L sodium sulfate was used as buffer; sample was injected at 50 mbar for 160 s under a separation voltage of -25 kV and at 25 $^{o}$C. Here, sodium sulfate in the buffer increases the conductivity of the buffer zone and provides sample stacking. Therefore, a high volume of injection was possible. Detection was done spectrometrically at 210 nm. The capillary was subsequently flushed for 2 minutes with 0.1 mol/L sodium hydroxide solution, water and the buffer between each run.

For the analysis of sugars, the capillary electrophoretic method developed for determination of carbohydrates was used. This method was successfully employed for pollen and honey samples (Kaygusuz et al. 2016, Kalaycıoğlu et al. 2017b) and other food samples (Kolayli et al. 2010, Kalaycıoğlu and Erim 2017) before. Here glycylglycine dipeptide is used as the seperation electrolyte, without the necessity to derivatize the analytes. Predefined optimal conditions were used as follows: 50 mmol/L glycylglycine at pH ~12.5. Samples were injected at 5 kPa for 6s from the anodic end under a separation voltage of 25 kV. Signal wavelength was 350 nm with a reference of 207 nm.

For all capillary electrophoresis analyses, the amounts of the analytes in the samples were calculated using calibration curves. All measurements were of at least triplicates.

### Antioxidant and antidiabetic activity

Antidiabetic and antioxidant activity measurements were carried out on a 96-well BioTek Power Wave XS microplate reader (Winooski, VT, USA).

α-Glucosidase enzyme inhibitory activities were studied by a slightly modified method of Shai et al. (Shai et al. 2011, Kalaycıoğlu et al. 2018). Here 50 μL of pH 6.8 phosphate buffer, 10 μL α-Glucosidase (0.5 U/mL in phosphate buffer) and 20 μL of diluted honey samples (10, 25, 50, 100 μg/mL) were preincubated for 15 min at 37 oC. After this step, enzymatic reaction was started by adding 20 μL of 5 mmol/L p-nitrophenyl-α-D-glucopyranoside substrate. After 20 minutes of incubation, reaction was stopped by adding 100 mmol/L of 50 μL Na2CO3 solution. Released p-nitrophenol was monitored at 405 nm using the microplate reader. Typical angiogenesis inhibitor, genistein, was also tested as the reference. The system without any test material was employed as the control.

The free radical-scavenging activities were studied by the modified DPPH assay (Blois 1958, Kalaycıoğlu et al. 2017a). This method is based on the monitoring the decreasing absorption of DPPH radical at 517 nm. 90 μL of 0.1 mmol/L DPPH solution was added to 10 μL sample solution and absorbance at 517 nm was measured 30 min later. Results were compared with the synthetic antioxidant BHA (Butylated hydroxyanisole). All calculations were done using the calibration curves for microplate readings.

## RESULTS AND DISCUSSION

Figure 1 shows the electropherogram of the honey sample for nitrate and nitrite analysis, where the upper electropherogram represents the sample spiked with 12.5 μmol/L standard nitrate and nitrite

and the lower electropherogram is the sample only. Calibration curves were plotted by the addition of acetonitrile (7.5% v/v) to the nitrite and nitrate standards. Correlation coefficient, limit of detection (signal-to-noise ratio: 3) and limit of quantification (signal-to-noise ratio: 10) were calculated as shown in Table 1.

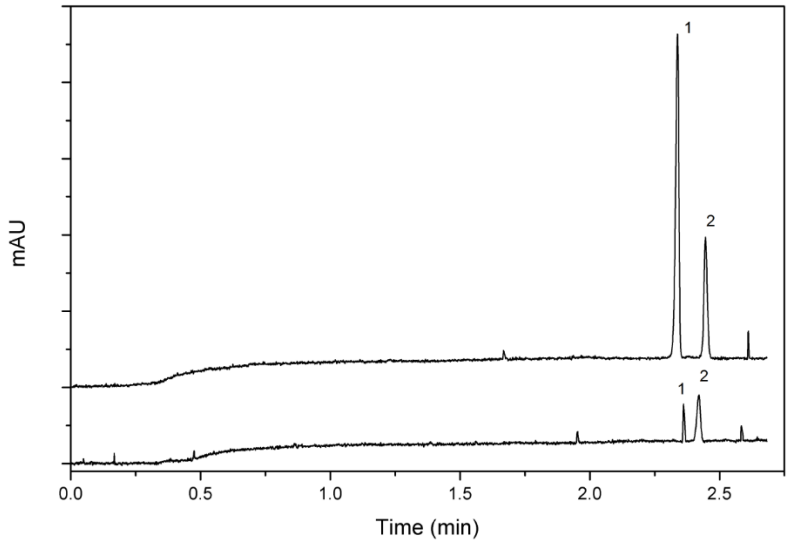


**Figure.1**. Electropherogram of the honey sample (bottom) and honey sample spiked with 12.5 µmol/L standard nitrate and nitrite solutions (top). Running potential: -25 kV, detection at 210 nm, under the buffer of 30 mmol/L formic acid, 30 mmol/L $Na_2SO_4$ and pH 4.0. Peaks 1: nitrate, 2: nitrite.

**Table 1.** Analytical parameters of nitrate and nitrite determination

| Parameter | Nitrate | Nitrite |
|---|---|---|
| Correlation coefficient of regression | 0.999 | 0.999 |
| Limit of detection, LOD (µmol/L) | 0.50 | 1.35 |
| Limit of quantification, LOQ (µmol/L) | 1.85 | 4.56 |

According to the results, nitrate and nitrate concentrations in the injected sample were found as 0.5597 ± 0.00174 µmol/L and 4,140 ± 0,123 µmol/L, respectively. These values are corresponding to 3.47 ± 0.01 mg/kg of nitrate and 19.05 ± 0.57 mg/kg of nitrite in the honey.

Figure 2 shows the electropherogram of the honey sample for glucose and fructose analysis. Correlation coefficient, LOD and LOQ for glucose and fructose analyses were listed in Table 2.

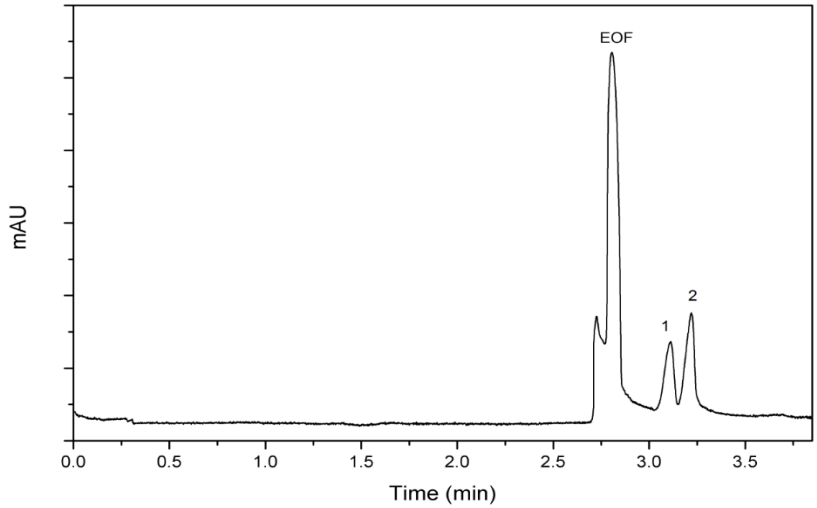


**Figure 2.** Electropherogram of the honey sample. Running potential: 25 kV, detection at 350 nm, under the presence of 50 mmol/L glycylglycine at pH 12.5. Peaks 1: EOF: electroosmotic flow, 1: glucose, 2: fructose.

**Table 2.** Analytical parameters of glucose and fructose determination

| Parameter | Glucose | Fructose |
|---|---|---|
| Correlation coefficient of regression | 0.995 | 0.991 |
| Limit of detection, LOD (mg/L, ppm) | 26 | 30 |
| Limit of quantification, LOQ (mg/L, ppm) | 96 | 111 |

According to the results, glucose and fructose concentrations in the injected sample were found as 418 ± 4 mg/g and 468 ± 3 mg/g of honey, respectively. Total amount of sugars in the honey sample was therefore 886 mg/g and fructose/glucose (F/G) ratio is 1.12.

α-Glucosidase inhibitory assay (IC50, in μg/mL) of the sample and genistein are found as 10.5 ± 0.56 and 5.50 ± 0.70 μg/mL, respectively. IC50 - DPPH of the sample is found as 74.48 ± 0.76 μg/mL.

**Table 3.** Results of α-Glucosidase inhibitory assay and DPPH.

| Analyte | $IC_{50}$ of α-Glucosidase inhibitory assay (μg/mL) | $IC_{50}$ for DPPH (μg/mL) |
|---|---|---|
| Honey sample | 10.5 ± 0.56 | 74.48 ± 0.76 |
| Genistein standard | 5.50 ± 0.70 | - |
| BHA standard[32] | - | 57.71 ± 0.55 |
| Multifloral honey from Hakkari [32] | - | 61.78 ± 0.56 |
| Citrus honey from Antalya [32] | - | 82.07 ± 0.96 |
| Clover honey from Diyarbakır [32] | - | 88.97 ± 0.98 |

Nitrite and nitrate are among the natural constituents of various food sources, namely vegetables and many others, as well as these are classified as food additivfundes for meat products (Kalaycıoğlu and Erim 2019). Nitrate and nitrite content of honey was previously evaluated (Beretta et al. 2010). It is reported that humans consume up to 8.7 mg of nitrite per person per day and the most of the consumption is related to dietary intake of cured meat (Anonymous 2003). Other nitrite-rich foods include vegetables such as spinach and lettuce. There is a concern on nitrite due to possible carcinogenic effects for over a long period, on the other hand, most nitrite is endogenously converted from nitrate, thus plays an important role in the nitric oxide (NO) metabolic product cycle (Ma et al. 2018) and recent reports indicate that there is no relation between nitrate and nitrite intake and stomach cancer (Bryan et al. 2012) as well as there is a beneficial side of the nitrates and nitrites in cardiovascular health (Lundberg et al. 2011). According to the results in the present study, the honey of Ombulak/Tuzluca region contains more nitrite than nitrate. Although the nitrite concentration much lower than vegetables such as spinach and lettuce (Iammarino et al. 2014) a honey sample with more nitrite than nitrate is not common.

Antioxidant activity of the honey is evaluated using the DPPH assay. According to the results, IC50 of the sample is found as 74.48 ± 0.76 μg/mL where the BHA is reported as 57.71 ± 0.55 μg/mL. When compared to Turkish honeys from very different locations (Kıvrak and Kıvrak 2017), Ombulak/Tuzluca honey is among the honeys have an upper-average antioxidant capacity. In addition to antioxidant characteristics, antidiabetic activity was found as 10.5 ± 0.56, which corresponds to 0.52 times the activity of Genistein standard, which indicates a good antidiabetic property. F/G ratio of the honey was found as 1.12, which indicates that the honey crystallizes slowly since F/G values above 1.0 indicate a slower crystallization (El Sohaimy et al. 2015, Radia et al. 2015). F/G ratio is typical for multifloral honeys and is within the quality limits of Turkish Food Codex Communiqué on Honey (1.0-1.4).

## CONCLUSION

This study reports an endemic honey from Ombulak, Tuzluca, Iğdır – Turkey. This region is a mountainous, sparsely populated and isolated from industrial presence. To the best of our knowledge, a honey from this region is reported for the first time. Results indicate that honey has a fair antioxidant

and antidiabetic character as well as a nitrite/nitrate ratio higher than 1.0. Although many kinds of honey have antidiabetic activity, as in the case of Iğdir honey, larger clinical studies are needed to make honey an alternative to sugar for diabetics. Further studies by medical scientists or biologists might reveal the biological characteristics of this type of honey.

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