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Investigation of Regional Variability in Chemical Composition of Turkish Honey through FTIR Analysis

Yeşim Kara[1]

[1]Pamukkale University, Department of Biology, Science Faculty, Denizli **Türkiye**

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***Correspondence:** E- mail: yesimopak@gmail.com Received: Oct., 02 2025, Accepted: Dec., 23, 2026, Revised: Jan., 20, 2026, Published online: Jan., 23, 2026.

The antioxidant properties of honey are primarily associated with its phenolic composition and are greatly influenced by its botanical and geographical origin. In this study, the total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity, and chemical characteristics of honey samples collected from different regions of Türkiye were determined using Fourier Transform Infrared (FTIR) spectroscopy and further evaluated through spectrophotometric analyses. Flower honey samples were obtained from Denizli (Servergazi/Sarayköy), Muğla (Doğançay), and Konya regions. TPC and TFC were determined using the Folin-Ciocalteu and aluminium chloride methods, respectively, while antioxidant activity was assessed by the DPPH radical scavenging assay. To identify functional groups related to carbohydrates and phenolic compounds, FTIR spectra were recorded in the 4000–400 cm^{-1} range. The FTIR analysis revealed characteristic absorption bands corresponding to O–H, C–H, and C–O vibrational modes and confirmed compositional differences between samples. The results demonstrate that FTIR spectroscopy is a meaningful complementary technique for interpreting antioxidant-related compositional differences in honey samples from different geographical origins.

Keywords: **Honey samples; total phenolic content (TPC); total flavonoid content (TFC); Apis mellifera L.; FTIR spectroscopy.**

INTRODUCTION

Honey is a natural food product composed primarily of sugars, predominantly fructose and glucose, along with minor constituents such as organic acids, proteins, enzymes, minerals, and phenolic compounds. The use of honey in Turkish culinary culture has been documented since the Seljuk period; however, historical sources indicate that its utilization became most widespread during the Ottoman era (Uslu, 2024). Other than its nutritional value, honey is used for pharmacological purposes. Researchers who have examined medical texts written in Old Uyghur and Chagatai have reported that Turks have used honey for medicinal applications since ancient times (Acar, 2021). It is known that Ibn Sina (Avicenna), the author of the renowned medical encyclopedia Al-Qanun fi al-Tibb (The Canon of Medicine), used honey mixtures to treat fever, pain, and wounds (Hançer & Yılmaz, 2019). Honey analyses have revealed a lack of data regarding the composition, total phenolic content, antioxidant activity, and phenolic profiles of anzer honey, making it quite difficult to determine its botanical origins (Çil, 2023). Today, honey is widely used not only for the prevention of various known ailments but also as a supportive therapeutic agent.

Regarding its demand at every stage of its existence, honey is extensively harvested and traded on a global scale. In this context, Türkiye ranks second worldwide in honey production after China (Kadiroğlu, 2024).

Although present in relatively low concentrations, phenolic acids and flavonoids play a crucial role in determining the antioxidant capacity and functional properties of honey. The antioxidant activity of honey has been widely investigated using radical scavenging and reducing power assays, with results demonstrating strong dependence on botanical and geographical origin. Variations in floral sources, soil composition, and climatic conditions lead to significant differences in phenolic profiles and, consequently, antioxidant behavior. Türkiye, characterized by high floral biodiversity, represents an important region for studying compositional diversity in honey.

Using this method, which is based on the ultraviolet–visible (UV–Vis) region of the electromagnetic spectrum, it is possible to analyze known natural products containing conjugated systems, aromatic groups, and functional groups such as >C=O, –N=O, –C≡N, –N=C=S, –N=N–, and $–SO_2–$ by UV–Vis spectrophotometry (Renuka et al., 2016).

There are many methods and popular analytical techniques, including as follows: mass spectroscopy and molecular spectroscopy (especially FTIR spectroscopy). The infrared spectroscopy technique is one of the most common analytical methods used to analyze honey nowadays. The main aim of the task was to use ATR-FTIR infrared spectroscopy to compare selected honey samples with typical spectral sequences from certain functional groups in the analyzed samples. In contrast, FTIR spectroscopy enables the analysis of natural products through characteristic absorption features associated with functional groups and molecular vibrations, including aromatic structures, N–H (NH_2), O–H, C=C, C≡C, C≡N, aldehydic C–H (H–C=O), carbonyl (>C=O), amide (H–N–C=O), C–O, and N–O stretching vibrations. While conventional antioxidant assays provide quantitative measurements of bioactivity, they do not directly reflect the molecular structure of honey constituents. Fourier Transform Infrared (FTIR) spectroscopy has gained increasing attention in food chemistry as a rapid, non-destructive technique for characterizing functional groups and molecular composition. In honey analysis, FTIR spectroscopy enables the identification of characteristic carbohydrate bands as well as signals associated with phenolic and organic acid components. This study was conducted to evaluate the bioactive contents of honey samples collected from different regions of Turkey and to assess these findings through FTIR-based chemical characterization. This study aims to determine and compare the physicochemical and biological characterization of some honey samples available in our country. Phenolic compounds were analyzed using the HPLC-DAD method. The total phenolic and flavonoid content (TPC, TFC), antioxidant capacity (DPPH), of the samples were determined, and FTIR analyses were performed. The spectra of the honey samples revealed characteristic bands corresponding to the functional groups of minor components.

MATERIALS AND METHODS

Honey Samples

Flower honey samples were collected from different geographical regions of Türkiye, including Denizli (Servergazi and Sarayköy), Muğla (Doğançay), and Konya. All samples were stored in airtight containers, protected from light, and prepared for subsequent analyses (Kara Y., 2021).

Determination of Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu method. Absorbance was measured spectrophotometrically, and results were expressed as milligrams of gallic acid equivalents per 100 g of honey (mg GAE/100 g).

Determination of Total Flavonoid Content

Total flavonoid content was measured using the aluminum chloride colorimetric method and expressed as milligrams of rutin equivalents per 100 g of honey (mg RE/100 g).

DPPH Radical Scavenging Activity

Antioxidant activity was evaluated using the DPPH free radical scavenging assay. Results were expressed as micromoles of Trolox equivalents per gram of honey (µmol TE/g).

FTIR Spectroscopic Analysis

FTIR (Fourier transform infrared) spectra were recorded using an ATR-FTIR spectrometer in the range of 4000–600 cm^{-1}. Spectral assignments were made based on characteristic absorption bands associated with carbohydrates, phenolic compounds, and other organic constituents.

RESULTS AND DISCUSSION

Previous studies have shown negative correlations between antioxidant activity values obtained from different tests, such as DPPH radical scavenging and FRAP tests. This inconsistency can be attributed to the different mechanisms of action of these methods, the selectivity of antioxidants, and the structural complexity of antioxidant compounds. While the FRAP test measures electron-donating ability, the DPPH test is based on the ability to capture hydrogen radicals. Consequently, some compounds may be highly effective at scavenging hydrogen radicals but have limited electron transfer capabilities (Floegel et al., 2011; Karadag et al., 2009). These differences are particularly evident in samples with complex chemical structures (Sánchez-Rangel et al., 2013). According to a study conducted by Malkoç et al., SC_{50} values ranging from 35.10 to 64.12 mg/mL were reported for the evaluation of antioxidant activity in anzer honey samples (Malkoç et al., 2019). The FTIR and UV-Vis spectra, which confirmed the chemical profile of the honey sample whose spectral findings they examined, revealed the corresponding bands for the functional groups of the honey's minor components.

Following our research and analysis, several important assessments can be made. It is known that the color of honey is closely associated with its flora or botanical origin. In some articles, this has been linked to acidity. In the present study, significant differences in total phenolic and flavonoid content were observed among honey samples. Muğla (Doğançay) honey has the highest phenolic content (402.45 mg GAE/100 g), while Konya honey has the highest flavonoid content (10.35 mg RE/100 g). These differences are consistent with regional differences in flora and nectar sources. The DPPH radical scavenging test revealed that Denizli (Servergazi) honey displayed the highest antioxidant activity (7.25 µmol TE/g) (Kara Y., 2021). This indicates that antioxidant capacity is not solely dependent on total phenolic concentration but is also

influenced by the specific composition of phenolic compounds and their synergistic interactions. Overall, among the samples reflected compositional differences that correlate with antioxidant activity. The samples were evaluated by FT-IR analysis, and the observed spectral variations are presented in Fig.1-2 and Fig.3-4.

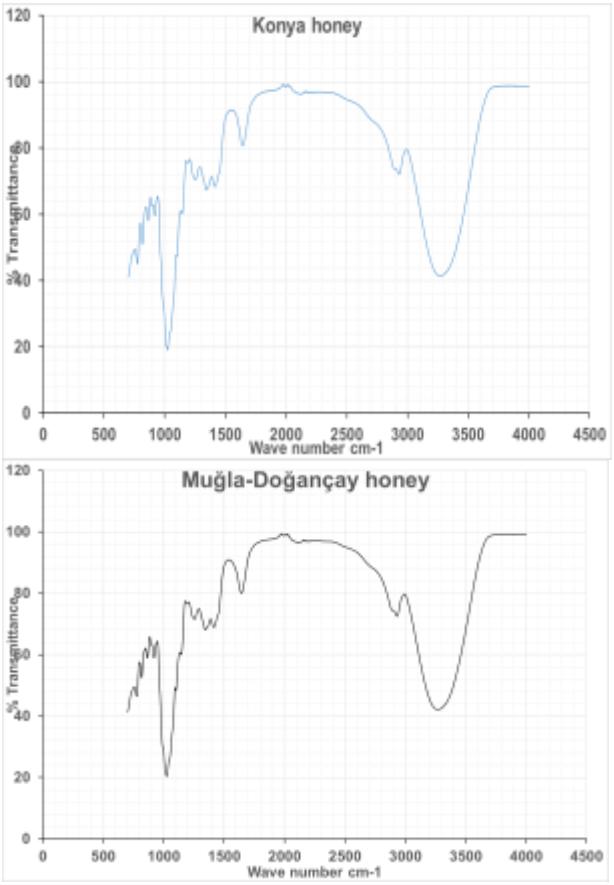

Fig. 1-2: FT-IR analysis of honey samples from Konya and Muğla-Doğançay, Türkiye. Wavenumbers are expressed in cm^{-1}, and peak intensities are denoted as s (strong), m (medium), and w (weak).

According to a previous study, FTIR analyses of honey samples revealed that all samples contained -OH (alcohol) and/or -NH (amine, amide) groups, aliphatic structures, aromatic systems, carbonyl-containing structures (aldehyde, ketone, carboxylic acid, amino acid, ester, amide, etc.), unsaturated structures (alkene, alkyne, polyene, carbonyl conjugated structures), and structures containing C-O and C-N.

In the present study, total phenolic content, antioxidant activity, and total flavonoid content were measured in four different honeys, and FTIR analysis was performed on the results obtained from the samples, revealing significantly different outcomes. These differences are attributed to the source and geographical origin of the honeys. The highest values were found in the data obtained from Servergazi honey from Denizli. For most physicochemical parameters, the differences in measured levels are dependent on the botanical origin of the honey.

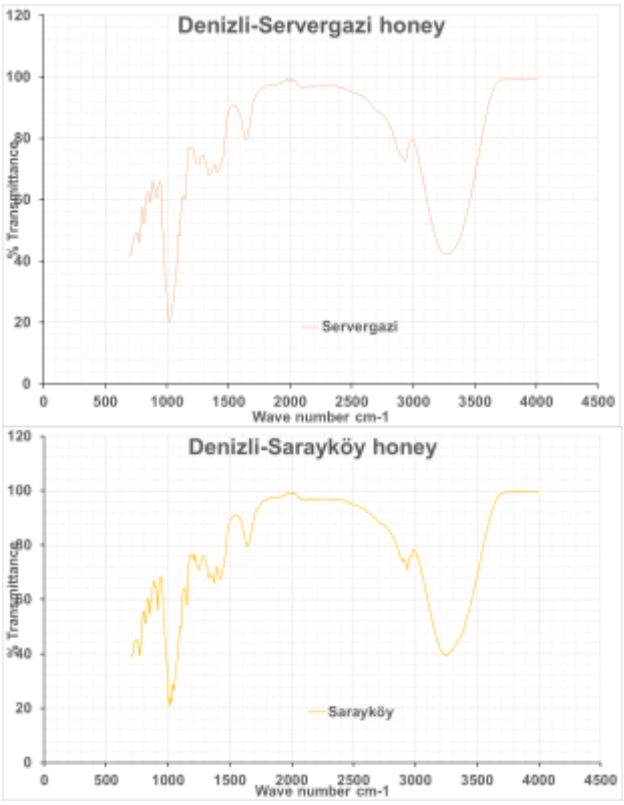

Fig. 3-4: FT-IR analysis of honey samples from Servergazi and Sarayköy, Türkiye.

In conclusion, the comparison of the phenolic profiles of a small number of honey samples suggests various markers, as indicated in the current literature. Moreover, when FTIR spectral data and DPPH results are evaluated together, it is shown that honeys with high antioxidant activity have a richer phenolic functional group profile, and that FTIR analysis is an effective tool for quickly and reliably predicting the antioxidant potential of honey samples.

CONCLUSION

The findings of this study demonstrate that honey samples from different regions of Türkiye exhibit distinct antioxidant capacities and chemical compositions. FTIR spectroscopy provided rapid and informative insights into structural differences associated with bioactive compounds and complemented conventional antioxidant assays. This integrated analytical approach is therefore well-suited for food composition studies and contributes to a better understanding of the chemical basis of honey's antioxidant properties. This study investigated the physicochemical and bioactive properties of honeys obtained from different regions of Türkiye. However, it is also important to consider the limitations of the study. The number of samples analyzed may not have been sufficient to fully represent

regional honey diversity and seasonal variations. In addition, the biological effects of these parameters on living organisms were not investigated.

CONFLICT OF INTEREST

The authors declared that the present study was performed in the absence of any conflict of interest.

DATA AVAILABILITY

All data are included within the article and on the hands of the first author if you need.

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AUTHOR CONTRIBUTION

Yeşim Kara: Conceived the idea, planned for the study, and writing the article.

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