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Some Quality and Compositional Characteristics of Flower and Pine Honeys from Different Geographical Regions

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ABSTRACT

The physicochemical properties and composition of honey may vary depending on its botanical and geographical origin, bee species, ratio of nectar, vegetation type, flowering period, foraging insect species, beekeepers' production techniques and timing of honey collection, and packaging and storage conditions of honey. In this study, some physicochemical properties of flower (F1, F2, F3 and F4) and pine (P1, P2, P3 and P4) honeys from different geographical origin (Antalya in Türkiye, Hessen in Germany and Lisbon in Portugal) were determined. The average °Brix, pH, and electrical conductivity (EC) values were 82.73, 4.28, and 0.44 mS/cm, respectively, with the ranges of 81.60-84.43 for °Brix, 3.79-4.85 for pH, and 0.15-0.68 mS/cm for EC. The color characteristics of honey were influenced by its botanical source and composition with the averages for CIE L*, a*, and b* were 24.29, 5.74, and 4.26, respectively. The flower and pine honey samples showed distinct UV-vis spectral profiles, especially in the 200-350 nm range, with differences attributed to their chemical composition, including sugar and phenolic contents. On the other hand, FTIR spectroscopy revealed similar spectral patterns for pine and flower honeys indicating shared functional groups and chemical structures in both honey types. Pine honey samples had significantly higher antioxidant activity values due to their phenolic contents ($p<0.05$). The highest antioxidant activity was found in the P4 sample, with 2.45 mmol TEAC/kg and 39.65% DPPH• radical inhibition ratio. Results indicated that the physicochemical and bioactive characteristics of flower and pine honey samples varied according to their specific botanical and geographical origins.

Keywords: Flower honey, Pine honey, Electrical Conductivity, Antioxidant Activity, FTIR

Farklı Coğrafi Bölgelere Ait Çiçek ve Çam Ballarının Bazı Kalite ve Bileşim Özellikleri

ÖZ

Balın fizikokimyasal özellikleri ve bileşimi, botanik ve coğrafi kökenine, arı türüne, nektar oranına, bitki örtüsü tipine, çiçeklenme dönemine, polen toplayan böcek türlerine, arıcıların üretim tekniklerine ve balın toplama zamanına, balın paketlenme ve saklanma koşullarına bağlı olarak değişebilir. Bu çalışmada, farklı coğrafi kökenlerden (Türkiye'de Antalya, Almanya'da Hessen ve Portekiz'de Lizbon) elde edilen çiçek (F1, F2, F3 ve F4) ve çam (P1, P2, P3 ve P4) ballarının bazı fizikokimyasal özellikleri belirlenmiştir. Ortalama °Brix, pH ve elektriksel iletkenlik (EC) değerleri sırasıyla 82.73, 4.28 ve 0.44 mS/cm olarak tespit edilmiş ve °Brix için 81.60-84.43, pH için 3.79-4.85 ve EC için 0.15-0.68 mS/cm aralıklarında değişim göstermiştir. Balın renk özellikleri, botanik kaynağına ve bileşimine bağlı olarak etkilenmiştir ve CIE L*, a* ve b* değerlerinin ortalamaları sırasıyla 24.29, 5.74 ve 4.26 olarak belirlenmiştir. Çiçek ve çam balı örnekleri, özellikle 200-350 nm aralığında, kimyasal bileşimleri (şeker ve fenolik içeriği dahil) nedeniyle farklılıklar gösteren belirgin

UV-vis spektral profiller sergilemiştir. Öte yandan, FTIR spektroskopisi, çam ve çiçek balları için benzer spektral desenler ortaya koyarak her iki bal türünde ortak fonksiyonel gruplar ve kimyasal yapılar bulunduğunu göstermiştir. Çam balı örneklerinde, fenolik içeriklerine bağlı olarak, önemli ölçüde daha yüksek antioksidan aktivite değerleri gözlemlenmiştir ($p<0.05$). En yüksek antioksidan aktivite P4 örneğinde bulunmuş olup, bu değer 2.45 mmol TEAC/kg ve %39.65 DPPH• radikal inhibisyon oranı olarak görülmüştür. Sonuçlar, çiçek ve çam balı örneklerinin fizikokimyasal ve biyoaktif özelliklerinin spesifik botanik ve coğrafi kökenlerine bağlı olarak değişiklik gösterdiğini ortaya koymuştur.

Anahtar Kelimeler: Çiçek balı, Çam balı, Elektriksel İletkenlik, Antioksidan Aktivite, FTIR

INTRODUCTION

Honey, a natural product that can sometimes crystallize, is collected by honeybees after they gather nectar from plants, secretions from living parts of plants, or secretions from plant-sucking insects. The bees then combine these substances with their own enzymes, modify them, reduce their water content, and store them in the honeycomb to mature [1]. Honey is a viscous fluid, partially or completely crystallized complex substance containing about 180-200 different types of components rich in components such as sugars (mostly fructose and glucose), organic acids, amino acids, enzymes, carotenoids, flavonoids, phenolic compounds, vitamins, minerals, aromatic compounds and pollen [1-4].

Honey is considered as a functional food which cannot be added to any food component or organic and/or inorganic substances that are not found in its natural composition, including food additives [1-5]. Besides the food industry, honey has been widely used in pharmaceutical and cosmetic fields since ancient times due to its taste, aroma and therapeutic properties (antibacterial, antiviral, anti-inflammatory and antioxidant activity, facilitating digestion and strengthening the immune system) provided by secondary plant metabolites such as flavonoids, polyphenols and volatile compounds, especially due to its antimicrobial effect in wound healing [3, 5, 6]. Although the chemical composition and physical properties of honey, such as color, aroma and flavor, and its bioactivity are influenced in a complex way by the plant species and source from which it is produced, nectar composition, climate, geographical source, harvesting techniques and storage conditions, it should have a unique odor and taste [3, 4, 7, 8].

Honey is usually divided into two types according to its source: flower honey and secretion honey. Nectar honey, also known as flower/blossom honey, is obtained from plant nectar (such as linden honey, clover honey, citrus honey, cotton honey, clover honey, thyme honey, heather honey, acacia honey and heather honey). In contrast, nectar/secretion honey primarily originates from the secretions of the living parts of plants or the secretions of plant-sucking insects (Hemiptera) striving on the living tissues of plants. Pine honey is a secretion honey produced by collecting and modifying the honeydew, which is a carbohydrate-rich honeydew of some pine trees (*Pinus brutia*, *P. nigra* and *P. pinea*), by honeybees during their developmental stages [1, 9].

The vast majority of the world's pine honey (more than 90%) is produced only in Greece and Türkiye, where the *Marchalina hellenica* insect living on *P. brutia* is located [10]. The color of secretion honey must be at least 60 according to the Pfund scale [9]. According to its botanical origin, honey can be called multifloral when produced from the nectar of several species or monofloral when it contains approximately 45% pollen from a dominant species [4]. Since the honey prices for some botanical and geographical origin are higher than others, it is important to ensure their authenticity. The location of Türkiye is highly suitable for honey production, which is the most in demand among bee products, the fact that the flowering period is spread throughout the year and that it has a rich flora that positively affects production can be considered as a great advantage for the country. When the increasing use of honey as an alternative to artificial sweeteners in recent years is taken into account, it becomes clear that the beekeeping market will continue to expand and grow even further. Türkiye is the second most important honey producer in the world after China. While the suitable flora and climate conditions in Türkiye allow beekeeping to be done economically, beekeeping stands out as an activity that can be done almost everywhere from sea level to high plateaus. With an annual honey production of approximately 100,000 tons, Türkiye has a favorable environment due to the abundance of flowers that provide an ideal environment for beekeeping activities [3].

The current research encompassed four floral honeys (F1-F4) and four pine honeys (P1-P4), which were available in local markets considering the geographical diversity and botanical origins of the honeys. Türkiye, as the second-largest global honey producer, with an annual output of approximately 100000 tons, boasts a favorable apicultural environment due to the abundance of flowers, which provides an ideal setting for beekeeping endeavors [3]. Antalya, Türkiye, offers a plethora of floral and pine honeys owing to its extensive altitude variation and Mediterranean vegetation. The most numerous beehives are in Spain, Romania, Poland, France, Italy, Hungary, Germany, Bulgaria and Portugal [11]. Five samples (F1, F2, P1, P2, and P4) were collected from this site. F3 and P3 from Hessen, Germany, are blended honeys obtained from both EU and non-EU nations, accessible in Central European markets. Moreover, Germany is the one of the top honeys producing country. F4, acquired from Burgos, Spain, purchased from Lisbon, Portugal, displays Mediterranean vegetation shaped by Atlantic influences. To facilitate a balanced assessment of botanical differences, each category (floral and pine honey) comprised four samples [11-13].

This study aimed to determine some physicochemical properties, color characteristics, and bioactive potential of flower and pine honey samples from different

geographical and botanical origins. In this study, the effects of factors such as total soluble dry matter, pH, electrical conductivity, total phenolic content, free radical scavenging activity, and spectral profiles in UV-vis and FTIR spectroscopy on the quality, authenticity, and potential health benefits of honey were evaluated.

MATERIALS and METHODS

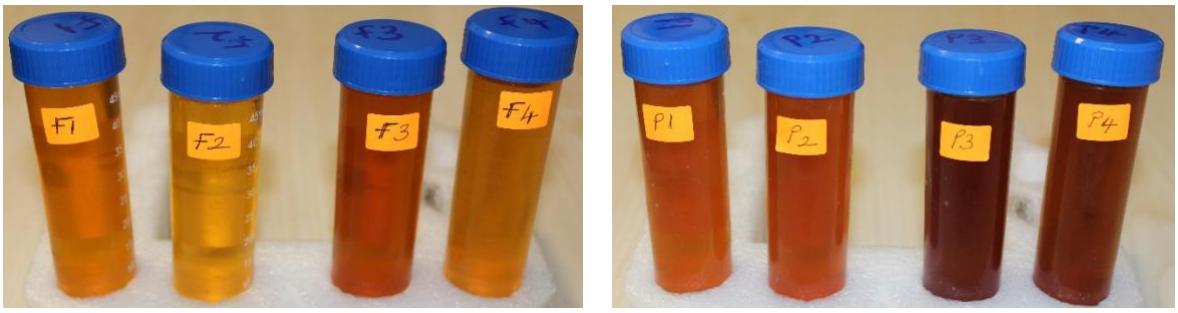
Materials

Flower honey samples (F1, F2, F3 and F4) and pine (secretion) honey samples (P1, P2, P3 and P4) were analyzed in the study. F1, F2, P1, P2 and P4 honey samples were obtained from different sales points in Antalya, Türkiye. F3 and P3 samples were purchased in Hessen, Germany while F4 honey sample were obtained in Lisbon, Portugal. All honey samples analyzed within the scope of this research are trademarked and were purchased in their original packaging from markets in the local markets. Some properties of the honey samples used in the study are summarized in Table 1, using the label information on them.

After the samples were obtained from different sales points, they were transferred to Burdur Mehmet Akif Ersoy University Scientific and Technology Application and Research Center and stored in a cool and dry environment prior to analyses. Figure 1 shows the distinct color differences of flower and pine honey samples obtained from different regions.

Table 1. Geographical origins and purchase details of honey samples selected for analysis

Type of Honey	Sample Code	City, Country of Purchase	Features	Packaging Type	Recommended Consumption Date
Flower	F1	Antalya, Türkiye	Mainly compiled from the plateaus and plains of Central and Southeastern Anatolia, Average altitude 500 m	Glass Jar	8.11.2026
	F2	Antalya, Türkiye	From the summit of the Taurus Mountains, 1500+ altitude	Glass Jar	26.05.2027
	F3	Hesse, Germany	A mixture of honey from EU and non-EU countries.	PET/PP	7.05.2026
	F4	Lisbon, Portugal	Origin: Burgos, Spain	Glass Jar	31.03.2027
Pine (Secretion)	P1	Antalya, Türkiye	Compiled from honey obtained mainly from Yerkesik and Marmaris regions.	Glass Jar	16.10.2026
	P2	Antalya, Türkiye	From Mediterranean pine forests, 100+ altitude	Glass Jar	23.05.2027
	P3	Hesse, Germany	A mixture of honey from EU and non-EU countries.	PET/PP	9.04.2026
	P4	Antalya, Türkiye	Prepared by collecting honey from pine trees in the Muğla region.	Glass Jar	3.08.2026



Flower Honey Samples Pine Honey Samples

Figure 1. Visual representation of the honey samples used in the study

Methods

Some chemical and physicochemical analyses were carried out based on literature research on the subject, honey standards and circulars.

Total Soluble Solids Content

The total soluble solids (°Brix) contents of honey samples were determined according to Bogdanov et al. [14] in triplicate. To determine the °Brix values of honey samples, a small amount of each sample was placed onto the prism of a digital refractometer (PAL-3, Atago Co. Ltd., Tokyo, Japan) at room temperature. °Brix values were obtained directly through the refractometer display and expressed as percentage (%). To determine the moisture content of honey, the °Brix values of honey samples were subtracted from 100.

pH Determination

The pH values of honey were determined by the Mettler Toledo Seven Multi (Mettler Toledo, Greifensee, Zurich, Switzerland) device. For this purpose, 10 grams of honey

was dissolved in 100 mL of ultrapure water, and then each sample was analyzed in triplicate at room temperature [14].

Electrical Conductivity

The EC values of honey were determined with the Mettler Toledo Seven Multi (Mettler Toledo, Greifensee, Zurich, Switzerland) device. For this purpose, 10 grams of honey sample was dissolved in 100 mL of ultrapure water and each sample was analyzed in triplicate, and mean EC values were expressed in mS/cm at room temperature [14].

Color Properties

The Commission International de L'Eclairage (CIE) L*, a* and b* color values of honey were measured in 3 replicates (each measurement with 3 light pulses) using a colorimeter (Konica Minolta Chroma Meter CR-400, Konica Minolta Inc., Tokyo, Japan), and the average L* (brightness value, 0 black, 100 white), a* [(+) redness, (-) greenness] and b* [(+) yellowness, (-) blueness) values were determined. Color analyses were performed using a D65 illuminator, 10° observer angle and 8 mm diameter diaphragm [15].

Determination of UV-vis Spectra

Honey samples (0.1 g each) were weighed into 15 mL Falcon® tubes, and 5 mL of distilled water was added before vortexing the mixture for 3 min. Solutions were subjected to ultrasonication in a water bath (WUCD06H, Daihan Scientific Co. Ltd., Gyeonggi-do, Korea) at 40°C and 100% power for 30 min to dissolve the crystals and achieve homogeneous sample solutions for spectrum measurements [16]. The UV-vis spectra of honey samples were recorded at wavelengths ranging from 200 to 600 nm using quartz cuvettes with UV-Vis spectrophotometer (Optizen Pop, Mecasys Co., Ltd., Daejeon, Korea). Subsequent to the acquired dilutions, measurements were performed at ambient temperature using distilled water as a blank reference.

Fourier Transform Infrared Spectral Analysis

The FTIR spectra of honey samples obtained with a spectrophotometer (Frontier Model Perkin Elmer, Waltham, MA, USA) equipped with a diamond single reflection attenuated total reflectance (FTIR-ATR) device. Each measurement was performed with 10 scans per spectrum at a spectral resolution of 4 cm^{-1} within the mid-infrared range, specifically from 4000 to 550 cm^{-1}.

ABTS Radical Scavenging Activity

Distilled water (5 mL) were added to each honey sample (0.5 g) and vortexed for 3 min to produce homogenous solutions (10%, w/v) for antioxidant activity and total phenolic content determinations. Subsequently, each solution was subjected to ultrasonication for 30 min at 40°C and 100% power in an ultrasonic water bath.

The ABTS assay was performed according to Re et al. [17] and Fratianni et al. [18] with slight modifications. The ABTS radical solution was prepared through the mixing of equal volumes of 7 mM ABTS aqueous solution and 2.45 mM potassium persulfate aqueous solution. The mixture was kept under dark at ambient temperature to generate the ABTS radical (ABTS+) for 12 to 16 h. The solution of ABTS radicals was diluted with chromatographic grade methanol to achieve a final absorbance of 1.000 at 734 nm. For the analysis, 200 µl of a 10% (w/v) honey solution or Trolox® solutions (for the calibration curve) were combined with 2,800 µl of ABTS radical and thereafter incubated in the dark for 30 min at room temperature. Absorbance measurements were performed against methanol at 734 nm. Results were expressed as µmol Trolox® equivalent antioxidant capacity (TEAC)/kg honey±standard deviation (SD).

DPPH Radical Scavenging Activity

The DPPH assay was carried out using the procedure described by Barbaric et al. [19]. For the analysis, 1.85 mL of methanol and 1.5 mL of DPPH solution (0.18 mM DPPH in methanol) were added to 0.15 mL of each honey solution (10%, w/v). Following a 30-min incubation period at room temperature in dark, absorbance values were determined at 517 nm against to pure methanol. The radical scavenging activity was determined using Equation 1:

$$\text{DPPH inhibition (\%)} = [(A_0 - A_1) / A_0] \times 100 \quad (1)$$

where A_0 is the absorbance of DPPH• solution, and A_1 is the absorbance of honey sample solutions.

Total Phenolic Content

The TPC values of honey were assessed using the Folin-Ciocalteu (FC) method as described by Tananaki et al. [20]. The FC reagent (2 N) was diluted with water to achieve a concentration of 0.2 N for analysis. A 0.5 mL honey solution (10%, w/v) was mixed with 2.5 mL of diluted FC reagent. After 5 min, 2 mL sodium carbonate solution (75 g/L) was added and vortexed. Following a 2-hour incubation period at room temperature in dark, absorbance values were determined at 760 nm. Results were presented in mg of Gallic Acid Equivalent (GAE) per kilogram of honey, as determined by the gallic acid calibration curve.

Statistical Analysis

The analysis of variance (ANOVA) was performed using Minitab19 software. Significant differences among the properties of honey samples were identified by the Tukey's multiple comparison test at a 95% confidence level ($p<0.05$). All analyses were carried out in triplicate.

RESULTS and DISCUSSION

Total Soluble Solids Content, pH and Electrical Conductivity of Honey Samples

The °Brix, pH and EC values of honey samples obtained from different sales points in Türkiye, Germany and Portugal are given in Table 2. The mean °Brix value of flower and pine honey samples purchased from different locations was calculated as 82.73 while mean pH was 4.28 and mean EC was 0.44 mS/cm. The ranges of °Brix, pH and EC values was found 81.60-84.43%, 3.79-4.85 and 0.15-0.68 mS/cm, respectively. According to the Turkish Food Codex Annex-1, the moisture content of flower and secretion honeys must be a maximum of 20% (minimum °Brix value of 80%). Results indicated that °Brix criteria were met in all honeys. In the Turkish Standards 3036 Honey Standard 4.2.2. Chemical Properties section, the pH value of honey is given as 3.4-6.1. This pH criterion was also met in all honeys. In the Turkish Food Codex Annex-1, the maximum EC for flower honey is presented as 0.8 mS/cm and the minimum EC for secretion honey is 0.8 mS/cm. EC criterion was met in all flower honey samples but not in secretory honey samples. There was statistically insignificant difference among the honey samples of F1, F4 and P3 and among the honey samples of F3 and P4 in terms of °Brix values. It was found that there was no statistically significant difference between honey samples coded F1 and F4, between honey samples coded F2 and F3, and among honey samples coded P1, P3 and P4 in terms of pH values. However, there was a statistically significant difference between flower honey and secretion honey in terms of pH values. There was statistically insignificant difference between F2 and F4 honey samples in terms of EC values. However, the difference in the EC values between the honeydew honeys was found statistically significant.

Table 2. Some physicochemical properties of honey samples

Sample Code	Total soluble solids (%)*	pH	Electrical Conductivity (mS/cm)
F1	$83.70\pm0.06^{BC**}$	3.96 ± 0.08^{C}	0.32 ± 0.003^{E}
F2	82.47 ± 0.25^{C}	3.80 ± 0.03^{D}	0.15 ± 0.000^{G}
F3	81.63 ± 0.15^{D}	3.79 ± 0.00^{D}	0.29 ± 0.001^{F}
F4	82.63 ± 0.38^{BC}	3.95 ± 0.03^{C}	0.15 ± 0.002^{G}
P1	83.10 ± 0.00^{B}	4.66 ± 0.01^{B}	0.64 ± 0.001^{C}
P2	84.43 ± 0.32^{A}	4.85 ± 0.01^{A}	0.66 ± 0.001^{B}
P3	82.87 ± 0.21^{BC}	4.61 ± 0.01^{B}	0.61 ± 0.002^{D}
P4	81.60 ± 0.10^{D}	4.58 ± 0.01^{B}	0.68 ± 0.001^{A}
Minimum	81.60	3.79	0.15
Maximum	84.43	4.85	0.68
Mean	82.73	4.28	0.44

*Values are given means±standard deviation. **Different superscript letters in the same column show significant differences ($p<0.05$).

The composition of honey is naturally influenced by various biotic and abiotic factors, including its botanical and geographical origin, climatic and seasonal conditions, beekeeping practices, and storage duration and conditions [4, 21]. The water content of honey, the second largest component, representing about 18% of its composition, is related to the maturity of honey. The moisture content is an important parameter that provides information about the quality and shelf life of honey, as it determines the stability and durability of honey and controls its resistance to spoilage due to yeast fermentation [5]. It also provides indirect information about the botanical and geographical origin of honey, the harvest season and storage conditions. According to the European Union and Codex Alimentarius regulations, the maximum moisture content of honey must be 20%, with the exception for heather honey (*Calluna vulgaris*), which can contain up to 23% water. Barbari et al. [16] determined the °Brix their honeydew samples ranged from 85.5 to 81.4, which complies with international standards. For instance, beech honeydew honey exhibited higher °Brix (85.5 and 85.4), while chestnut honey samples had lower values (81.4 and 82.5). These results were consistent with the literature. Mahani et al. [22] determined the °Brix content of *A. dorsata* honey samples ranged from 82.00 to 81.33. Santos et al. [23] investigated the physicochemical properties, chemical composition, and antioxidant activity of Australian stingless bee honey from two species, *Tetragonula carbonaria* (TC) and *T. hockingsi* (TH), across different times of the year. TC in May 2022 °Brix 73.0±0.153; in September 2022 °Brix 28.0±0.0577; in November 2022 °Brix 73.5±0.000. TH in May 2022 °Brix 30.0±0.0577; in September 2022 °Brix 70.9±0.0577; in November 2022 °Brix 72.9±0.0577. In general, honey produced in the northern hemisphere contains many propolis flavonoids like pinocembrin, pinobanksin and chrysin originating from the native poplars whereas honey from equatorial regions and Australia is usually lacking those compounds due to the absence of poplars [24]. Schmidt et al. [24] collected leatherwood honey samples from 2020 (n=52) and 2021 (n=55) were kindly supplied by 12 Tasmanian apiarists from a total of 81 beehive sites as unpacked, raw honeys. The division of samples into ‘North’ and ‘South’ was based on a geographical barrier. Schmidt et al. [24] determined 2020 North honey samples °Brix 84.3±0.5, 2020 South honey samples °Brix 84.1±0.8; 2021 North honey samples °Brix 84.0±0.9, 2021 South honey samples °Brix 84.0±0.8. In total, five Centauri® honey samples were collected in August 2022 in different regions of Türkiye [3].They found °Brix A 86±1, B82±1, C 84±1, D 85±1, E 83±1. In an investigation on the physicochemical parameters of raspberry, rosehip, alfalfa, hawthorn, polyfloral and honeydew honey samples from Bucovina, Romania, and of manuka honey samples were analyzed to characterize the honey

samples and verify their usefulness in classifying honey depending on the botanical origin. The °Brix values were reported as Hawthorn 80.97±2.04, Alfalfa 81.27±2.30, Rosehip 82.01±2.12, Honeydew 83.29±1.26, Manuka 80.71±0.95, Polyfloral 81.85±1.58 and Raspberry 81.39±2.22 [14]. Four groups of honeys of various origins such as monofloral honey (MF), blossom honey (BL), acacia honey (AC), and mountain blossom honey (MBL) were collected between August and September 2022 from various region of Kosovo [7]. They determined MF °Brix 81.11±1.46, BL °Brix 81.17±1.51, AC °Brix 79.94±2.38 and MBL °Brix 80.57±0.45. In the study conducted by Binici et al. [25] by obtaining 15 flower honey samples from beekeepers in five different locations of Erzurum in the 2022 flower season, the °Brix value varied between 76.80-84.23.

Honey is acidic because it contains organic acids like gluconic acid and inorganic ions such as phosphates and chlorides [5, 7]. The pH value of honey is a basic indicator of its acidity, which affects both its stability and preservation [19]. The natural acidity of honey typically varies between pH 3.5 and 5.5 [5, 14]. In the TS 3036 Honey Standard [9] 4.2.2. Chemical Properties section, the pH value of honey should be between 3.4 and 6.1. Studies have shown that there is a positive correlation between EC and pH values since the ion concentration in honey affects both parameters [16]. Our results supported this connection, as the honey samples analyzed exhibited higher EC and pH levels. Beech honeydew honey had a pH of 5.52 to 5.43, while chestnut honey samples showed pH values of 5.10 to 5.41 [19]. Mahani et al. [19] found *A. dorsata* honey samples exhibited pH values between 3.90 (Siak) and 4.43 (Ternate). Siak honey had the lowest pH, followed by Bandung (4.25) and Ternate. The average pH ranged from 3.61 for hawthorn honey to a maximum of 4.14 for honeydew honey. They determined pH values in Hawthorn, Alfalfa, Rosehip, Honeydew, Manuka, Polyfloral and Raspberry honey samples as 3.61±0.26, 3.78±0.52, 3.89±0.30, 4.14±0.21, 3.73±0.12, 4.04±0.40, and 3.50±0.11, respectively [5]. Santos et al. [3] investigated the physicochemical properties, chemical composition, and antioxidant activity of Australian stingless bee honey from two species, TC and TH, across different times of the year. TC in May 2022 pH 3.73±0.027; in September 2022 pH 3.77±0.006; in November 2022 pH 3.93±0.021. TH in May 2022 pH 3.57±0.045; in September 2022 pH 4.14±0.010 and in November 2022 pH 4.19±0.027. Schmidt et al. [24] reported the pH values of honey samples as follows: In 2020, the North honey samples exhibited a pH of 4.92±0.15, whereas the South honey samples displayed a pH of 5.15±0.48. In 2021, the North honey samples had a pH of 4.83±0.26, and the South honey samples showed a pH of 5.13±0.38. Filipe et al. [3] documented the pH values of five Centauri® honey samples -A, B, C, D, and E- with corresponding pH values of 4.35, 3.05, 3.10, 3.20, and 3.60, respectively. Koraqi et al. [7] evaluated the pH of various honeys, reporting values of 4.00±0.24 for MF, 3.94±0.12 for BL, 4.12±0.10 for AC, and 3.77±0.16 for MBL. Binici et al. [25] determined pH values, with a range of 3.55 to 4.19.

EC, which measures the ability of organic and inorganic substances to ionize and conduct electricity, depends on the botanical origin of the honey and the concentration of ionizable minerals and the amount of dissociated acids in its content. Since EC is the main feature that distinguishes flower honeys from secretion honeys, it is used as a reliable and important parameter to distinguish honey types and to ensure their quality and originality [5, 10, 19]. According to the Turkish Food Codex [1], the EC in flower honeys should not exceed 0.8 mS/cm, and the EC of secretion honeys should not be lower than 0.8 mS/cm. This difference is due to the fact that the two types of honey have different chemical compositions due to the fact that secretion honeys are produced by insects that directly suck the phloem of the plant, which is richer in minerals than flower nectar, with a higher content of minerals and organic acids that contribute to the increase in electrical conductivity In routine honey quality controls, the EC value is used to distinguish between secretion honey and flower honey, replacing the ash content determination over time [19, 26].According to Schmidt et al. [24], Northern honey samples had EC 541±21 µS/cm, whereas Southern honey samples had EC 559±68 µS/cm. In 2021, Northern honey samples had EC 490±26 and Southern honey samples had 516±25 µS/cm. Filipe et al. [3] found that the EC values of five Centauri® honey samples were 1.65±0.21, 0.44±0.02, 0.31±0.00, 0.22±0.00, and 0.72 µS/cm. According to Koroqi et al. [7], honey EC values ranged from 0.29 to 0.39 (mS/cm) in different types of honey. Barbarić et al. [19] determined the EC values for beech and chestnut honeydew honey between 1.25 and 1.65 mS/cm. Ninety-five honey samples in total gathered from various Greek geographical regions [8], and EC (mS/cm) values were determined as 0.53±0.26 in blossom, 0.73±0.08 in acacia, 1.26±0.17 in chestnut, 0.35±0.10 in thyme, 0.33±0.10 in orange, 0.60±0.06 in cotton, 0.91±0.09 in arbutus, 0.81±0.26 in heather, 1.21±0.17 in fir, 1.02±0.20 in pine, and 0.94±0.20 in oak honey samples. A total of 156 commercial honey samples, classified as thyme, pine, or polyfloral, were gathered from five Mediterranean countries, namely Greece, Malta, Spain, Tunisia, and Türkiye [10]. The median EC values were similar across the four countries, with Tunisia, Spain, Greece, and Türkiye showing values of 348, 397, 381, and 356 µS/cm, respectively. Mahani et al. [22] reported the EC values of honey between 0.56 to 3.32 mS/cm. Luca et al. [5] determined the EC values of honey in mS/cm, presenting the following averages with standard deviations: 323.78±125.31, 395.66±132.46, 463±124.42, 990.15±84.29, 482.33±44.43, 578.52±136.69, and 384.25±193.97.

Color Parameters of Honey Samples

The CIELAB color parameters of flower and pine honey samples are given in Table 3. Also in Figure 2, some differences in the colors of honey samples can be seen. There was a wide diversity in the L*, a* and b* values of honey because the pollen used by bees to make honey was obtained at different times, at different altitudes and from different sources (flower and pine secretion).

Table 3. CIELAB color values of honey samples used in the study

Sample Code	L* (Lightness)	a* (Redness)	b* (Yellowness)
F1	25.26±0.10^{C**}	9.18±0.02^{B}	6.80±0.12^{B}
F2	29.93±0.04^{A}	10.26±0.10^{A}	12.33±0.33^{A}
F3	23.12±0.15^{F}	5.09±0.06^{C}	2.28±0.09^{C}
F4	25.77±0.10^{B}	9.07±0.33^{B}	6.93±0.14^{B}
P1	23.46±0.04^{E}	4.13±0.02^{D}	1.83±0.04^{D}
P2	23.95±0.07^{D}	4.04±0.12^{D}	1.01±0.07^{E}
P3	22.31±0.17^{G}	1.90±0.02^{E}	0.87±0.04^{E}
P4	20.52±0.03^{H}	2.26±0.03^{E}	2.01±0.01^{CD}
Min.	20.52	1.90	0.87
Max.	29.93	10.26	12.33
Mean	24.29	5.74	4.26

* L*: light (100) to dark (0), a*: red (+a*) to green (-a*), b*: blue (-b*) to yellow (+b*). **Values are given means±standard deviation, and different superscript letters in the same column show significant differences ($p<0.05$).

The averages of the L*, a* and b* color values were calculated as 24.29, 5.74 and 4.26, respectively (Table 3). The ranges for the L*, a* and b* values of honey samples were found 20.52-29.93, 1.90-10.26 and 0.87-12.33, respectively. The differences in the L* and b* color values of honey samples were found statistically significant ($p<0.05$). Conversely, there was no substantial variation in a* values across F1 and F4, P1 and P2, and P3 and P4. Upon assessment of b* values, no statistically significant variation was observed between honey samples F1 and F4, as well as between samples P2 and P3. It was concluded that a statistically significant difference existed between blossom honey and secretion honey regarding their b* values.

The color of honey, which is a natural, nutrient-rich and functional food, is considered one of the most important characteristics in terms of commercial aspects, as it greatly affects its quality and consumer preferences [22, 27]. The color of honey depends on many substances that affect its chemical composition, including metal content, phenolic compounds, flavonoids, pigments such as chlorophyll and carotenoids [26,27]. Minerals, in particular, can significantly affect its color by forming complex reactions with other compounds in honey [26,27]. It was also found that the color of honey is positively correlated with antioxidant activity [26,27]. The color of honey is an important parameter in the identification of single-flower honeys because it is related to botanical species [26, 27]. The color of honey is classified according to lightness (L*) values, ranging from white or pale yellow to amber or black [22, 26, 27]. Dark-colored honeys may have higher metal concentrations compared to light-colored ones while dark colors (chestnut and nectar honey) are associated with high metal concentrations such as Cd, Fe and Pb, while light-colored ones (eucalyptus and thyme) are associated with Al and Mg [19]. Undesired changes in the color of honey may be due to incorrect beekeeping practices, exposure of honey to light during storage and production a long time ago [26]. In a study on the color characteristics of six honey varieties (jujube, linden, buckwheat, acacia, lychee, and vitex) from China, a comparable lightness (L* ~25.9) was noticed in jujube and linden honeys; however, the lowest L* (25.41) and a* (0.24) were recorded in buckwheat honey. Acacia honey was found to have the maximum lightness (28.99) and a moderate a* value (1.15), but lychee and vitex honeys displayed elevated a* values of 2.07 and 2.34, respectively [27]. The floral source of each honey was shown to be responsible for its unique color characteristics.

UV-vis Spectra of Honey Samples

Spectroscopic methods such as UV-vis, FTIR, near-infrared (NIR) and Raman spectroscopy can be utilized to identify the authentication of honey. These techniques have been reported to be effective in detecting honey adulteration, geographical and botanical origin when coupled with chemometrics. The UV-vis spectra within the 200-600 nm range of flower and pine honey samples from the different geographical origins are depicted in Figure 2A. The 200-400 nm range of the UV-vis absorption spectra provide critical analytical insights into the composition of honeys. The absorbance band ranging from 200 to 260 nm mostly corresponds to glucose, fructose, and phenolic chemicals. Amino acids like tryptophan, proteins, and phenolic chemicals exhibit absorbance at 260–300 nm, whereas the 300–340 nm region is associated with flavonoids and other phenolic compounds [16, 28].

The flower (Figure 2B) and pine (Figure 2C) honey samples displayed variations in spectral profiles, particularly in the 200-350 nm region. The variations were attributed to the chemical composition of honey samples, specifically the sugar profile and phenolic content. Blossom honeys (e.g. flower) have been reported to possess a higher sugar content and a lower phenolic concentration compared to honeydew honey (e.g. pine) [16]. In current study, the absorption bands of phenolic compounds in pine honey were more intense, as seen in Figure 2C. Therefore, the color of pine honey depicted in Figure 1 is darker, more likely due to the increased phenolic content.

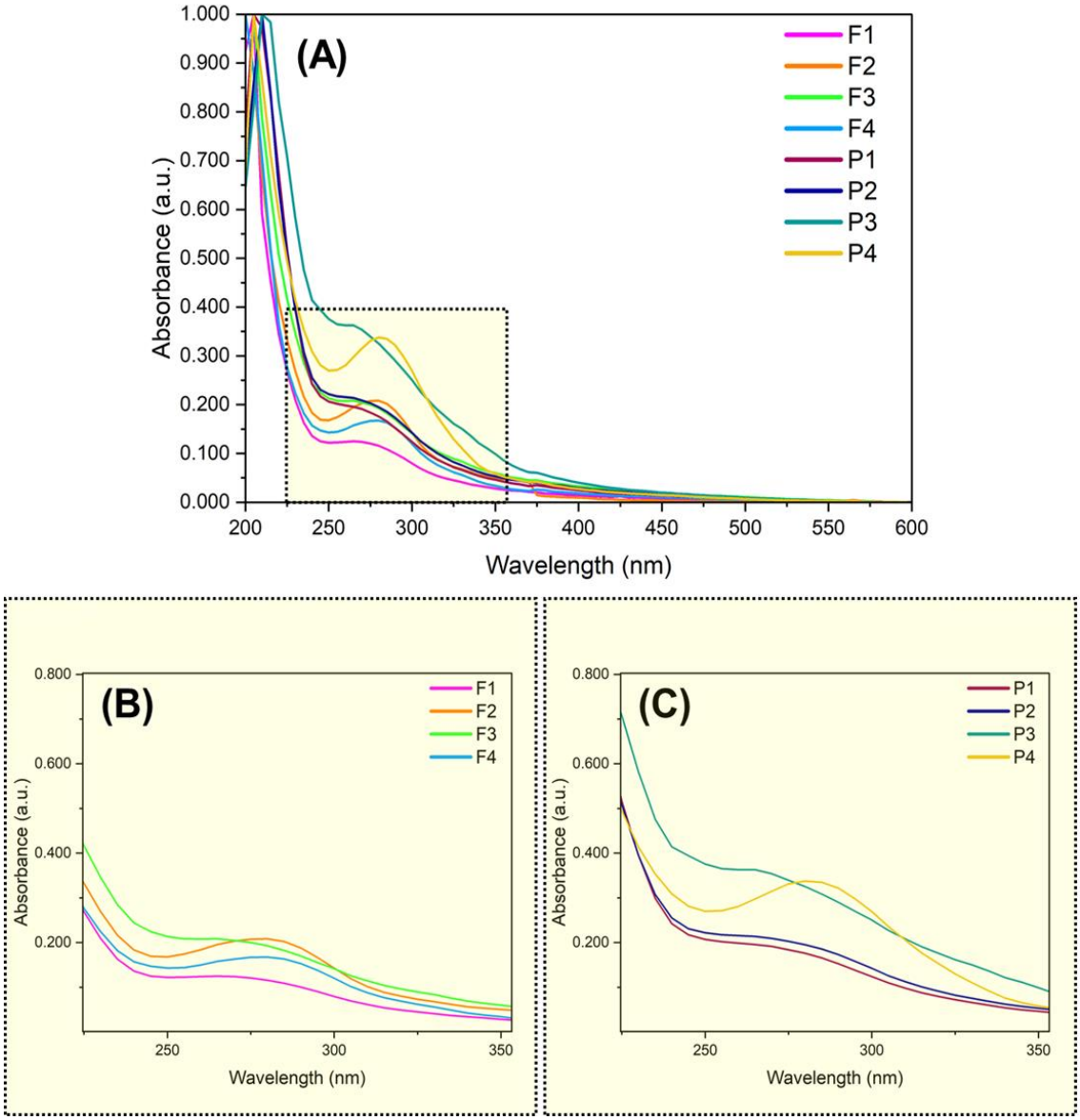


Figure 2. (A) UV-vis spectra of honey samples in the range of 200-600 nm, (B) The expanded range of UV-vis spectra of flower honey samples (F1-F4) between 225-355 nm, and (C) The expanded range of UV-vis spectra of pine honey samples (P1-P4) between 225-355 nm.

FTIR Spectroscopy

FTIR spectroscopy provides a molecular fingerprint by detecting IR absorption at certain frequency ranges that represent mechanical movements of functional groups [29]. The FTIR spectra of honey samples in the medium infrared region between 4000 cm^{-1} and 550 cm^{-1} are presented in the Figure 3A. The FTIR spectra of pine and flower honeys had a similar pattern, signifying the existence of analogous functional groups and chemical structures in both types. The honey spectra exhibited a pronounced peak at 3280 cm^{-1} associated with –OH stretching. The peak at 2920 cm^{-1} is attributed to C–H stretching associated with carboxylic acids and the $–NH_3$ stretching band of free amino acids present in low concentration within the composition of honey [5, 30]. Tightly bound water exhibited absorption at 1641 cm^{-1}, indicative of H–O–H bending (–OH deformation), and a tiny amount of protein molecules (N-H bending of amide I) and carbohydrates (C-O vibrations) also contributed to the absorption intensity in this location [5, 30]. The peak at around the wavenumber of 1342 cm^{-1}, indicative of amino acids and proteins, corresponds to the C–H deformation vibration coupled with the C–N stretching of amide III [5, 30].

The most sensitive absorption zone of honey's main components is the band, called as fingerprint region, below 1500 cm^{-1} which is the best region to quantify honey sugars and organic acids (Figure 3B). The broad absorption band located between 1180-950 cm^{-1} is associated with C–O stretching vibration of carbohydrates, while the peaks at 1148 cm^{-1} (sucrose), 1097 and 1049 cm^{-1} (glucose and fructose) and 983 and 965 cm^{-1} (fructose) denote the characteristic bands for honey sugars. The absorption band with a peak about 920 cm^{-1} relates to the C–H bending of the carbohydrate [30-32]. The region under the 900 cm^{-1} is known as

indicative of the saccharide configuration. The spectral region of 898-837 cm^{-1} includes markers linked to C–H deformation vibrations of α-pyranose and C–H deformation bands of β-pyranose [29]. At last, the peak at 775 cm^{-1} is ascribed to markers that may arise from C–H out-of-plane deformation vibrations of aromatic compounds or C–S stretching bands from certain organic sulfur compounds [32].

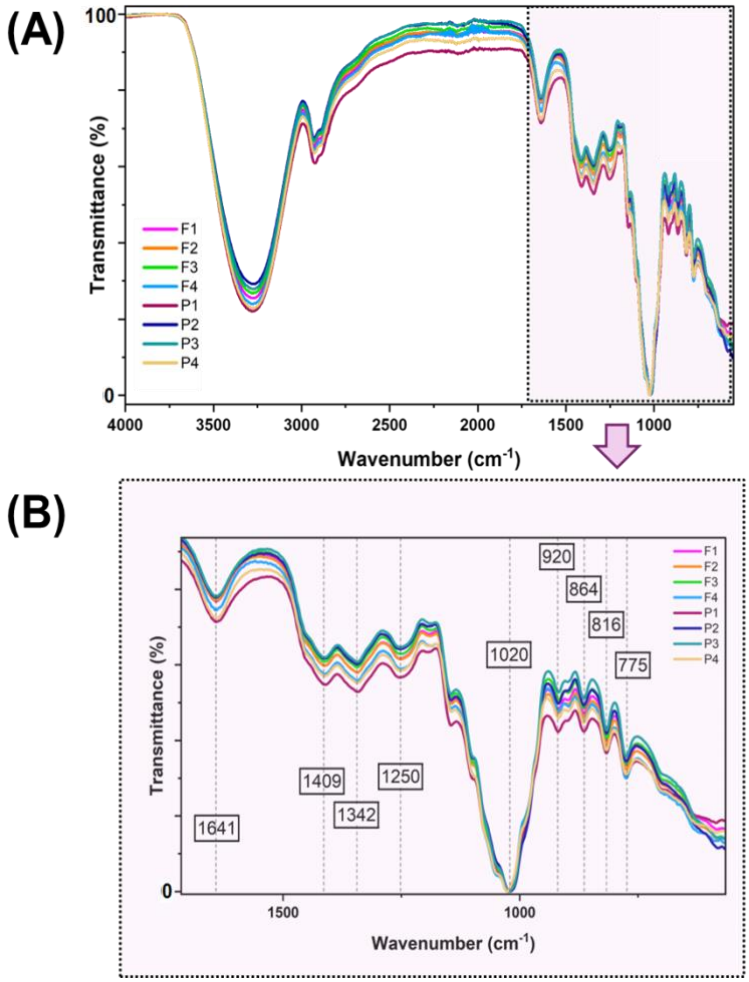


Figure 3. (A) FTIR spectra of honey samples (F1-F4: Flower honey, P1-P4: Pine honey) in the range of 4000-550 cm^{-1}. (B) The expanded range of FTIR spectra of honey samples between 1700-550 cm^{-1}.

Antioxidant Activity and Total Phenolic Content of Honey Samples

The antioxidant activity of honey samples from different geographical origin was evaluated using both DPPH and ABTS radical scavenging assays, and results are summarized in Table 4. Pine honey samples showed significantly higher antioxidant activity values compared to flower honey samples, which might result from the variations in their phenolic contents ($p<0.05$). The highest antioxidant activity, regarding both assays, was observed for the P4 sample as 2,448.85 µmol TEAC/kg honey and 39.65% inhibition of DPPH• radical. Honeydew honey (from pine, oak, willow, Abies, and Picea) exhibits distinct differences from floral honeys in both physical and biological characteristics. Because, honeydew honey is derived from the byproducts of aphid digestion that honeybees gather from plant phloem, whereas blossom honey originates from the nectar of flowering plants [34-36].

The botanical and geographical origins of honey are the primary factors influencing the variation in phenol contents [37]. The antioxidant properties of honey arise from a variety of constituents, including phenolics, peptides, organic acids, enzymes, minerals, and Maillard reaction products [38]. The effectiveness of these compounds in neutralizing free radicals mainly depends on their chemical structure, particularly the ability of phenolic compounds to transfer electrons or hydrogen ions [39]. As reported in many studies, darker-colored honeys usually have higher phenolic contents, which is strongly associated with increased antioxidant activity [32, 33]. In this study, the total phenolic content of honey samples showed a high correlation coefficient with the L* ($r= -0.810$), a* ($r= -0.902$), and b* ($r= -0.703$) color values, ABTS ($r= 0.977$) and DPPH ($r=0.897$) assays according to the Pearson correlation test ($p<0.05$).

Table 4. Antioxidant activity and total phenolic content of honey samples

Sample Code	ABTS•+ radical scavenging Activity* (µmol TEAC**/kg honey)	DPPH• inhibition (%)	Total phenolic content (mg GAE****/kg honey)
F1	916.20±21.79^{E***}	15.85±0.31^{D}	407.32±10.62^{C}
F2	733.44±63.17^{E}	12.73±0.46^{D}	400.00±13.90^{C}
F3	1,291.49±114.98^{D}	14.32±0.25^{D}	635.84±10.77^{B}
F4	787.19±38.90^{E}	13.64±0.63^{D}	407.31±10.53^{C}
P1	1,466.30±15.20^{CD}	19.14±0.74^{C}	596.54±12.77^{B}
P2	1,580.25±95.81^{C}	20.41±1.13^{C}	642.16±2.83^{B}
P3	2,232.72±87.37^{B}	27.05±1.83^{B}	1,010.84±35.64^{A}
P4	2,448.85±52.37^{A}	39.65±2.02^{A}	1,048.45±66.83^{A}

*Values are given means ± standard deviation. **TEAC: Trolox Equivalent Antioxidant Capacity; ***Different superscript letters in the same column show significant differences ($p<0.05$). ****GAE: Gallic Acid Equivalent.

Among the flower honey samples, F3 honey had the highest total phenolic content (635.84 mg GAE/kg honey) and ABTS•+ radical scavenging activity (1,291.49 µmol TEAC/kg honey) although the difference was insignificant at DPPH radical inhibition rates (12.73-15.85 %). Otmani et al. [33] reported that monofloral and polyfloral honey samples exhibited DPPH inhibition ranging from 6% to 47% and total phenolic content between the 640-1900 mg GAE/kg honey. The total phenolic content of pine honey was found as 614.2 mg GAE/kg honey, according to a study conducted in Türkiye by Can et al. [36]. Moreover, the total phenolic content of 20 pine honey samples collected from Mugla and Marmaris (Türkiye) varied from 620.1 to 687.8 mg GAE/kg of honey [40]. Together with the results of the current study, these results indicate the intricate relationship between honey's chemical composition and its antioxidant capability, providing important insights into how origin and botanical source affect its nutritional and functional qualities.

CONCLUSION

The Codex Alimentarius and the European Union Directive specify that honey could be labeled by its botanical origin if it predominantly originates from that source and displays the distinctive microscopic, physicochemical, and organoleptic attributes. Therefore, assessing honey's quality and authenticity is crucial for ensuring consumer confidence in regional honey and preserving authentic honey production practices. The physicochemical characteristics and bioactive properties may play a key role in determining the botanical origin of honey. The botanical origin can lead to variations in pH and EC values, with flower honey samples exhibiting lower pH and reduced EC values. Conversely, pine honey samples from various geographical origins exhibited significant differences based on these factors ($p<0.05$). Differences in the total phenolic contents of honey may lead to variations in its antioxidant activity and color properties. Pine honey, for instance, demonstrated higher antioxidant activity and a darker color compared to floral honey. The °Brix, pH, and EC values of the honey samples were found in good consistent with the standards set by the Turkish Food Codex, with floral honeys meeting the EC limit, while secretion honey samples detected lower than the level it. The UV-vis and FTIR spectra of honey provided valuable information on its botanical origin and chemical composition. These results indicated how botanical source and geographical origin might influence the chemical and bioactive properties of honey, offering valuable insights into its potential applications in both food and medicinal fields. This investigation is constrained by the restricted sample size and geographical variety. Furthermore, environmental factors such as climate, harvesting methods, storage conditions, and seasonal variations, which have significant effects on honey composition, were not comprehensively evaluated. Future research should include a broader range of honey kinds from diverse botanical and geographical origins, while also considering seasonal and environmental influences. Additionally, the utilization of advanced methods of analysis (chromatographic techniques, NMR spectroscopy, DNA barcoding, chemometrics and machine learning) may improve the precision of botanical origin identification and augment comprehension of the bioactive constituents in honey.

CONFLICT of INTEREST

The authors declare no conflict of interest.

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