Recep Tayyip Erdoğan Üniversitesi Fen ve Mühendislik Bilimleri Dergisi, 4 (1) 51-64 2023
Recep Tayyip Erdogan University Journal of Science and Engineering, 4 (1) 51-64 2023
ISSN: 2687-2315 / E-ISSN: 2757-7686

Melissopalynological and Physico-Chemical Properties of Cimil Plateau (Rize) Honey

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Araştırma Makalesi/Research Article
Geliş Tarihi/Received: 28.11.2022
Kabul Tarihi/Accepted: 23.06.2023

ABSTRACT

In this study, melissopalynological and physico-chemical properties of the honey produced in the Cimil Plateau (Rize province) were examined in detail. Examined honey samples were taken from eight different beehives in Cimil Plateau from different altitudes and localities. All samples were analyzed by standard laboratory methods to determine moisture, sugar, proline, diastase, and 5-Hydroxymethyl furfural (HMF) parameters. Also, melissopalynological analyses were made according to the standard techniques and determined pollen composition of the honey samples. Melissopalynological studies showed that the pollens belonged to Asteraceae, Brassicaceae, Campanulaceae, Caprifoliaceae, Caryophyllaceae, Cistaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Lauraceae, Liliaceae, Onagraceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae and Tiliaceae families. In the sample number 6, *Castanea sativa* pollens which are dominant were found in proportion to 70%. The moisture content of the samples was determined to be 16.9-19.1 %, and the proline content was found to be 568-758 mg/kg. In sugar analysis, it was determined that Fructose was 35.36-51.4 g/100 g and Glucose was 21.35-65.78 g/100 g. According to the results, the examined honey samples are polyfloral and monofloral. In this study, different honey samples taken from Cimil Plateau have been investigated for the first time in detail in terms of their melissopalynological and physico-chemical properties. It has been observed that the melissopalynological and physico-chemical analysis results of honey samples examined are in accordance with European Union Standards and Turkish Food Codex Honey Communiqué.

Keywords: Cimil Plateau, Honey, Melissopalynology, Rize

Cimil Yaylası (Rize) Balının Melissopalinolojik ve Fiziko-Kimyasal Özellikleri

ÖZ

Bu çalışmada, Cimil Yaylası (Rize)'nda üretilen balların melissopalinolojik ve fiziko-kimyasal özellikleri detaylı olarak incelenmiştir. İncelenen bal örnekleri Cimil Yaylası'ndan farklı yükselti ve lokalitelerden olmak üzere 8 farklı kovandan alınmıştır. Tüm numuneler nem, şeker, prolin, diyastaz ve 5-Hidroksimetili furfural belirlemek için standart laboratuar yöntemleriyle analiz edilmiştir. Ayrıca standart tekniklere göre melissopalinolojik analizler yapılmış ve bal örneklerinin polen kompozisyonları tespit edilmiştir. Melissopalinolojik çalışmalarda polenlerin Asteraceae, Brassicaceae, Campanulaceae, Caprifoliaceae, Caryophyllaceae, Cistaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Lauraceae, Liliaceae, Onagraceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae ve Tiliaceae familyalarına ait olduğu görülmüştür. *Castanea sativa* polenleri 6 numaralı bal örneğinde %70 oranında dominant olarak bulunmuştur. Örneklerdeki nem miktarı 16.9-19.1, prolin miktarı ise 568-758 mg/kg olarak belirlenmiştir. Şeker analizlerinde Fruktozun %35.36-51.44 g/100 g ve Glukozun 29.34-45.7 g/100 g arasında değiştiği tespit edilmiştir. Elde edilen sonuçlara göre incelenen bal örneklerinin polifloral ve monofloral olduğu belirlenmiştir. Bu çalışma ile Cimil Yaylası'ndan alınan farklı bal örnekleri ilk kez melissopalinolojik ve fiziko-kimyasal özellikleri bakımından detaylı olarak incelenmiştir. İncelenen balların melissopalinolojik ve fiziko-kimyasal analiz sonuçlarının Avrupa Birliği Standartları ve Türk Gıda Kodeksi Bal Tebliği'ne uygun olduğu görülmüştür.

Anahtar Kelimeler: Cimil Yaylası, Bal, Melissopalinoloji, Rize

Cite as;
Yetkin, G., Kundakçı, S., Kanbur, E.D., Coskunçelebi, K., Makbul, S. (2023). Melissopalynological and Physico-Chemical Properties of Cimil Plateau (Rize) Honey, *Recep Tayyip Erdogan University Journal of Science and Engineering*, 4(1), 51-64. Doi: 10.53501/rteufemud.1209594
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1. Introduction

Honey is defined as the sweet substance that honeybees store in honeycombs after collecting the nectars of flowers, the secretions of plants or some living things living on plants, and then mixing and modifying their specific substances. Although most of its content is sugar, it does not only consist of sugar, but also contains nutritious and medicinal properties (Özgüven et al., 2020). There are approximately 200 different components in the structure of honey. Due to their vitamins, minerals, organic acids, flavonoids, phenolic acids, amino acids, and enzymes it contains, honey is a functional food that is easy to digest, nutritious and has protective and therapeutic properties against many diseases (Özmen and Alkın, 2006; Spilioti et al., 2014).

The chemical composition of honey varies according to its geographical and botanical origin. However, the honey basically consists of macro and micro components such as 82% carbohydrates, 17% water, 0.7% minerals, 0.3% protein, vitamins, organic acids, phenolic compounds, and free amino acids. The main sugars in honey are fructose and glucose, and besides these monosaccharides, it also contains disaccharides such as sucrose, maltose, isomaltose, lactose, galactobiose, and some oligosaccharides (Islam et al., 2012; Moniruzzaman et al., 2014). Flora of Türkiye forms a bridge between the Floras of Southern Europe and Southwest Asia. Therefore, it is among the richest regions of the world in terms of plant biodiversity, after the equatorial and subequatorial zones (Çobanoğlu, 2012). There is a close relationship between the country's climate, topography, hydrographic conditions, and beekeeping. The high diversity of plants directly affects bee health and its products (Tunçel, 1992). A strong bee colony and a flora rich in nectar plants are the most critical factors affecting honey yield (Polat et al., 2020). Melissopalynological analysis of honey is an effective method for providing information about the origin, geographical structure, and quality of the nectar plants sourcing honey. In the following years, melissopalynological examinations of the honey of Türkiye continued (Yurtsever and Sorkun, 2005; Kaya et al., 2005; Erdoğan et al., 2009; Ünal and Sağlam, 2009; Taşkın and İnce 2009; Demir, 2013; Çelemli et al., 2018; Özler, 2018). Similarly, the melissopalynological properties of honey belonging to different plateaus and districts of Rize Province the area of this study were also investigated (Demir, 2011; Derebaşı et al., 2014; Malkoç et al., 2019; Çelemli et al., 2021; Kanbur and Atamov, 2022). Kanbur and Atamov (2022) have been stated that palynological and chemical properties of Ayder (Çamlıhemşin/Rize) honey. Their results revealed that the families of Apiaceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Cistaceae, Ericaceae, Fabaceae, Fagaceae, Geraniaceae, Lauraceae, Pinaceae, Rosaceae and Tiliaceae had the common Plant taxa similar to findings of Derebaşı et al. (2014), Çelemli et al. (2021) and Kanbur et al. (2021). According to TÜİK (2017), the Eastern Black Sea region is the second largest producers of honey in Türkiye.

The research area, including Cimil Plateau and its surroundings, is located within the borders of the İkizdere district of Rize Province. Bayburt and Trabzon Provinces are in the west, Erzurum Province is in the South, Kalkandere and central Rize districts are in the North, and Çayeli and Çamlıhemşin districts are in the east. İkizdere district has a classic oceanic climate with almost no drought season. The annual rains are 2254.4 mm, and the annual average temperature is 14.3 ˚C. The coldest month is January at 6 ˚C, and the hottest one is July at 24 ˚C (Anşin, 1981; Akman, 1995). In addition, it is seen that the study area differs in terms of plant diversity and vegetation types (Figure 1). The Eastern Black Sea region has a rich floristic structure (Ulusoy, 2010). There have been some floristic studies in Rize and in neighboring areas (Güner et al., 1987; Terzioğlu, 1998; Çobanoğlu, 2012; Demir, 2013). It has been determined that the flora of Tunca Valley Natural Park (Ardeşen/Rize) and its environs includes 1756 plant specimens (Baykal et al., 2018). Similarly, Baykal (2019) studied flora in the region Kaçkar Mountains National Park

(Başhemşin/Rize) and its regions contains Euro-Siberian 247 (49.1%), Irano-Turanian 17 (3.4%), Mediterranean 6 (1.2%) phytogeographic elements taxa. The Flora of the area between Galerdüzü and Çeymakçur Plateaus (Çamlıhemsin/Rize) was investigated (Demir and Atamov, 2019). As a result of the study, it was determined that the region endemism ratio was 8.84% (Demir and Atamov, 2019). Also, the families which are the richest in terms of the taxa number are as follows Asteraceae (25 taxa), Rosaceae (16 taxa), Lamiaceae (12 taxa) and Poaceae (9 taxa), respectively (Demir and Atamov, 2019). Cimil Plateau has an important with magnificent nature, unique architecture, thermal spa, climatical conditions and floral abundance. Also, Cimil Plateau is in the same region as the famous Anzer Plateau, which is known for its honey of commercial importance. It is seen that Anzer honey has been studied a lot in terms of flora, melissopalynological, physio-chemical and antioxidant content. However, it is seen that the honey samples of Cimil Plateau have never been investigated. The aim of this study was to determine the melissopalynological properties and physico-chemical changes of honeys produced with different types of flora in the Cimil Plateau in the very humid north-eastern Black Sea region.

Figure 1. Vegetation types in Cimil Plateau: (a) Forest, (b) Subalpine (photographed by Makbul)

2. Materials and Methods

2.1. Collection of Honey Samples

Eight honey samples were taken from Cimil Plateau, Rize Province (Figure 2) from different altitudes and localities. For example, honey samples used in the study were collected from beekeepers in Ortaköy (3, 4, 5, 7), Güvenköy (1, 6), Başköy (2) and Meşe (8) localities (Table 1).

The distribution of beehives at different elevation levels in the research area was determined by a layered random sampling method. A total of 500 g honey samples were taken from randomly selected beehives in the 2021 harvest season, placed in sterilized jars, and taken to the laboratory. Eight honey samples brought to the laboratory for analysis were kept at 18 ◦C (Kanbur et al., 2021).

2.2. Melissopalynological Analysis

According to Louveaux et al. (1970), a total of 10g of stock honey samples thoroughly mixed with a sterile glass rod were taken and transferred to the test tube, and then 20 mL of distilled water was added. For dissolution of the honey sample in pure water, the tubes were placed in a water bath at about 45 ◦C for 30–45 min, and then each tube was shaken by a stirrer. The solution was then centrifuged at 3500 rpm for 45 min, and the supernatant was poured off. The precipitate remaining at the bottom of the tube was infused with a quantity of basic fuchsin-added glycerin-gelatin, and this material was then transferred onto a glass slide. The slide was heated at 30–40 ◦C to allow the dissolution of the basic fuchsine, and glycerin gelatin was added. Then, an 18x18 lamella was placed on top. Then, the prepared slide was examined using light microscopy

(Nikon Eclipse E100). Photographs were taken using Bs200pro Image Processing and Analysis software at 40x and 100x magnification (Louveaux et al., 1970).

2.3. Physico-Chemical Analysis of Honey Samples

The physical parameters moisture (%), HMF, proline, diastasis, and sugar were analyzed to determine the quality of the honey subject to research in agreement with the European Union (Bogdanov et al., 1997). Honey sample moisture analyses were done by a portable refractometer and determined as % ratio (Bogdanov et al., 1997; Devillers et al., 2004; TS 13359, 2008). For moisture content, 1 g of each sample was dried to constant weight in hot air oven at 70°C, and the moisture was calculated on dry basis. Accordingly, 1 g honey sample taken with the help of a glass baguette was transferred to the glass chamber of the refractometer, and the % moisture content was determined by looking through the lensed part **(**Devillers et al., 2004).

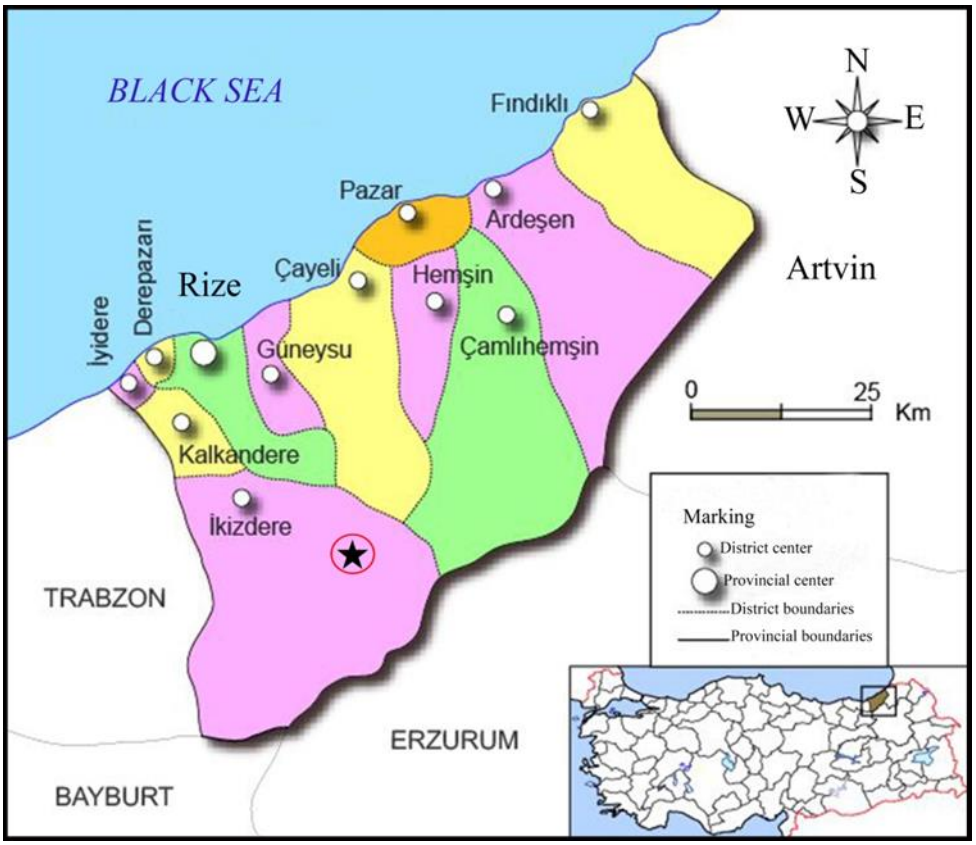

Figure 2. Location of the Rize in Türkiye (★: Cimil Plateau; Saygılı, 2020)

Table 1. Location information of the examined honey samples

Sample No	Locality	Altitude	Latitude and Longitude
1	Rize: Cimil Plateau, Güvenköy	1920 m	N 40° 44′ E 40° 45′
2	Rize: Cimil Plateau, Başköy	2180 m	N 40° 43′ E 40° 49′
3	Rize: Cimil Plateau, Ortaköy	2170 m	N 40° 43′ E 40° 49′
4	Rize: Cimil Plateau, Ortaköy	2170 m	N 40° 43′ E 40° 49′
5	Rize: Cimil Plateau, Ortaköy	2170 m	N 40° 43′ E 40° 49′
6	Rize: Cimil Plateau, Güvenköy	1920 m	N 40° 44′ E 40° 45′
7	Rize: Cimil Plateau, Ortaköy	2170 m	N 40° 43′ E 40° 49′
8	Rize: Cimil Plateau, Meşe	1786 m	N 40° 44′ E 40° 43′

The honey samples were analyzed by High Performance Liquid chromatography with UV detector reversed phase column (IHC, 2009). One gram of honey samples was diluted up to 50 ml with distilled water, filtered on 0.45 lm filter, and immediately injected in a HPLC (Varian 9012Q) equipped with a Diode Array Detector. The prepared mobile phase was placed in the HPLC device and flowed through the column with a flow rate of 1 ml/min for conditioning. Viale samples were injected into the conditioned HPLC system. HMF was identified by splitting the peak in honey with a standard HMF (Anonymous, 2009c). HPLC conditions for HMF analysis in honey are detailed in Table 2.

Table 2. HPLC conditions for HMF analysis in honey

Colon	Reverse phase C-18 Colon (125x4mm, 5 µm)
Colon temperature	25 ºC
Mobile phase	Water-Methanol (90: 10, v/v)
Flow rate	1ml/dak.
Injection time	15 dak.
Injection volume	20 µl
Wavelength	285 nm

Proline content was calculated by using a color comparison after applying ninhydrin with a proline standard. Samples for proline analysis were prepared by following Meda et al. (2005). The content was expressed as a proportion of the mass of honey tested. A 0.5 mL solution of honey was mixed with one ml of formic acid (80%) (Merck, KGaA, Darmstadt, Germany), one ml of ninhydrin (Merck, KGaA, Darmstadt, Germany) solution (3% in an ethylene glycol monomethylether (Merck, KGaA, Darmstadt, Germany) and shaken powerfully for 15 min.

The mixture was placed in a 70 ˚C bath for 25 min. A five ml solution of 50 %2-propanol (Merck, KGaA, Darmstadt, Germany) in pure water was then added, and the mixture was left to cool for 45 min at room temperature. Samples were measured on a microplate reader spectrometer (Thermo) at a wavelength of 510 nm. Water was used as the blank, and 0.032 mg/ml solution of proline (Merck, KGaA, Darmstadt, Germany) was used as the standard solution. The mean of three readings was used (Ough, 1960).

Diastase activity was determined using 10 g of honey weighted, and then 5 mL of acetate buffer was added, together with 15 mL of water. When the sample was completely dissolved, 3 mL of sodium chloride the solution was diluted to 50 mL with water. Moreover, a starch solution was standardized using an iodine solution. Reading against starch was made with a Thermo brand spectrophotometer at a wavelength of 660 nm. The analysis results were evaluated according to the Turkish food codex honey communique (communique no: 2020/7; Bogdanov et al., 1997).

The sugar analysis was done by High Performance Liquid Chromatography (HPLC) with RID detector and by using a carbohydrate column. A total of 5 g of honey was weighed in a 100 mL beaker. By adding 40 mL of distilled water and mixing it with a glass baguette, the honey was dissolved in the water thoroughly. Total sample solution 25 mL of methanol was added to the mixture and made up to 100 mL with distilled water. The sample, which was drawn from the mixture with a syringe with 1.5 cc and filtered through a 0.45 µm membrane filter, was taken into a vial and injected into the conditioned HPLC system. A standard chromatogram was created using fructose and glucose (Sigma Aldrich, USA) standards (TS 13359, 2008).

3. Results

3.1. Melissopalynological Analysis

As a result of the melissopalynological analysis of the honey samples from Cimil Plateau in İkizdere district of Rize Province, 8 genera and 3 taxon

belonging to 18 families were determined. Melissopalynologic studies showed that the pollens dominantly belonged to Fagaceae (*Castanea sativa*) and Fabaceae (*Onobrychis* sp.) families. In honey samples collected from the belt above the forest, pollens belonging to Asteraceae, Brassicaceae, Campanulaceae, Caprifoliaceae, Caryophyllaceae, Cistaceae, Ericaceae, Fabaceae, Lamiaceae, Lauraceae, Liliaceae, Onagraceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae and Tiliaceae taxa were found in rare and minor ratios (Table 3). Also, due to the lack of all plants have reference preparations, a species level evaluation could not be made. Our findings mostly indicated the diversity in the floral origin of samples. In addition, the honey samples 1, 2, 3, 4, 5, 7, 8 were identified as polyfloral and 6 was identified as monofloral (*Castanea sativa*). The appearance of some pollen under a light microscope and some families and photos are given in Figure 3 and 4.

Table 3. Pollen spectrum of Cimil Plateau honeys (D; > 45 % dominant pollen, S; 16-44 % secondary pollen, M; % 3-15 minor pollen, E; < % 3 rare pollen)

Pollen type	**Honey Sample Numbers**							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Asteraceae	E	-	-	-	-	-	E	M
Brassicaceae	M	-	-	M	E	-	-	-
Campanulaceae								
Campanula sp.	S	M	-	M	-	-	-	-
Caryophyllaceae	-	-	-	E	-	-	-	-
Caprifoliaceae								
Cephalaria sp.	-	-	-	-	-	-	-	E
Cistaceae								
Cistus sp.	-	E	E	E	E	-	-	E
Ericaceae	S	E	-	M	E	-	E	E
Fabaceae						-		
Onobrychis sp.	S	M	M	M	E	E	M	S
Trifolium sp.	S	M	M	M	E	E	M	S
Fagaceae								
***Castanea sativa* Miller**.	-	-	-	-	-	**D**	-	-
Lamiaceae	S	M	E	E	-	E	-	M
Teucrium sp.	E	-	-	-	-	-	E	M
Lauraceae								
Laurus nobilis L.	-	-	-	-		-	-	E
Liliaceae	-	-	-	-	E	-	E	E
Onagraceae								
Epilobium sp.	-	-	-	-	-	-	E	S
Poaceae	-	-	-	-	-	-	-	E
Polygonaceae								
Rumex sp.	M	M	E	-	-	-	-	-
Ranunculaceae	-	-	-	-	-	-	-	S
Rosaceae	S	S	E	E	-	E	-	-
Tiliaceae								
Tilia rubra DC.	S	S	E	-	E	-	E	S
Unidentified	E	-	-	-	-	-	-	-

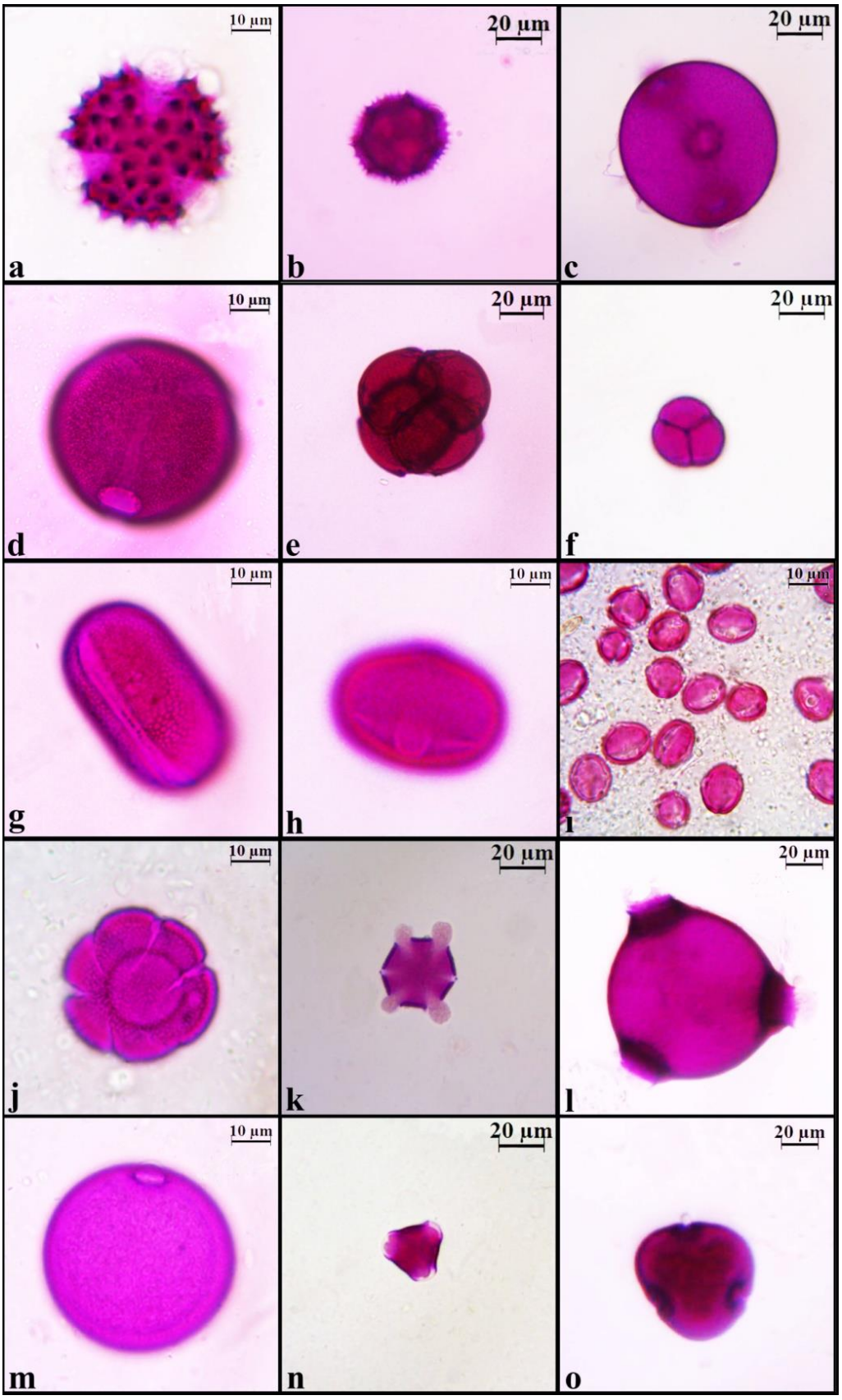

Figure 3. Pollen photos of some melliferous plants, which are determined in the studied area: a-b. (x100; x40) Asteraceae, c. (x40) Caryophyllaceae, d. (x100) Cistaceae, e-f. (x40) Ericaceae, g-h.(x100) Fabaceae, ı.(x100) Fagaceae, j-k. (x100; x40) Lamiaceae, l. (x40) Onagraceae, m. (x100) Poaceae, n. (x40) Rosaceae, o. (x40) Tiliaceae.

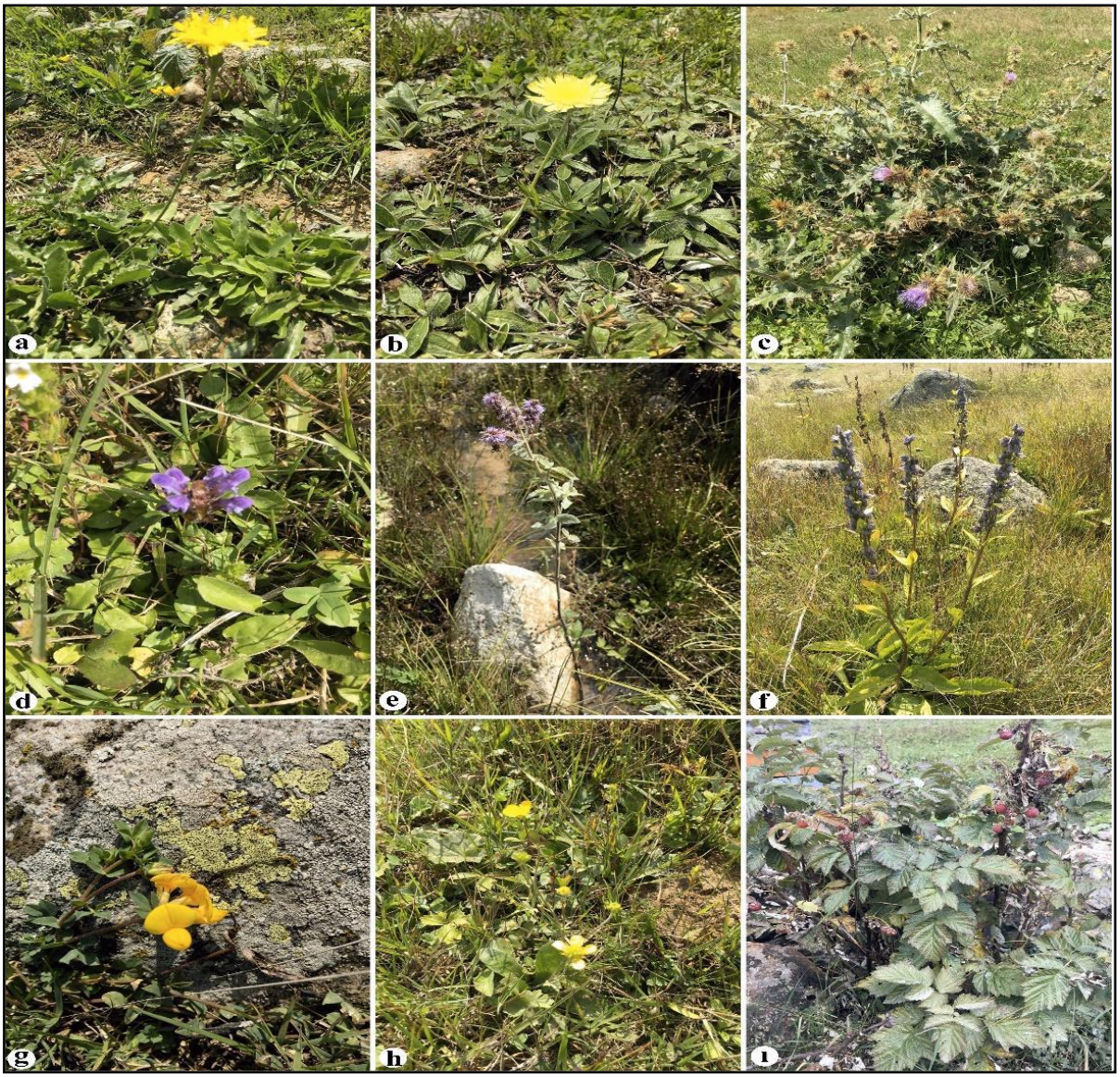

Figure 4. Images of Asteraceae (a-c), Lamiaceae (d-f), Fabaceae (g), Ranunculaceae (h), Rosaceae (ı) from the habitat (photographed by Makbul).

3.2. Physico-Chemical Analysis

The moisture, HMF, proline, diastasis and sugar of honey samples are showed in Table 4. HMF values of the honey samples 3, 4, 5, 7, 8 could not be measured because it was below the detection limit. It was determined that the findings obtained as a result of the physico-chemical analysis were in accordance with the Turkish Food Codex Honey Communique (2020/7).

Table 4. Physico-chemical results. *n.d.: non-defining; S: sample.

	S1	S2	S3	S4	S5	S6	S7	S8
Moisture	17.4	17.4	19.1	17.1	19.2	18	16.9	17.2
HMF	0.70	0.24	n.d.	n.d.	n.d.	2.29	n.d.	n.d.
Proline (mg/ 100 g)	616.29	587.85	568.88	758.51	568.88	616.29	635.25	597.33
Diastasis (%)	9.01	16.51	13.00	12.52	37.61	53.95	29.67	20.77
Fructose (%)	40.64	37.98	51.44	44.42	40.12	47.7	40.24	35.36
Glucose (%)	31.14	29.4	40.86	33.34	31.22	34.34	30.23	45.7
Sucrose (%)	0.2	0.16	-	-	-	-	0.19	-

4. Discussion

In this study, melissopalynological and physico-chemical analysis of honey taken from 8 different points belonging to the Cimil Plateau and from the İkizdere district of the Rize Province was carried out. The role of honey in protecting human health is closely related to its purity and composition, and it varies depending on many factors, such as the geographical structure of the region, flora, harvest time, and production method (Özbalci et al., 2013; Hotaman, 2015). According to food codex's (such as TSE), the purity and naturalness of honey are determined by using criteria such as water content, conductivity, acidity, pH, color value, sugar ratios (%C4/C13), proline amount, diastase activity, hydroxymethylfurfural (HMF) value, and insoluble substances (White, 1994). In the present study, pollen diversity and physical properties of honey, such as HMF, proline, diastase, sugar, and moisture contents, were determined.

The biological value of honey comes from its secondary metabolic products which are approximately 1% of total content (Weston and Brocklebank, 1999). An important part of these metabolites consists of a boundless number of aromatic and aliphatic compounds synthesized by plants. Most of the secondary metabolites with aromatic structures are phenolic compounds (Ulusoy, 2010). Therefore, in this study, the phenolic compound content of honey produced in the Cimil Plateau, which has a very rich flower flora, was also studied. As a result of current study, they were determined as *Campanula* sp., *Cephalaria* sp., *Cistus* sp., *Epilobium* sp., *Laurus nobilis*, *Onobrychis* sp., *Rumex* sp., *Teucrium* sp., *Tilia rubra* and *Trifolium* sp. genera, which belong to the Asteraceae, Brassicaceae, Campanulaceae, Caprifoliaceae, Caryophyllaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Lauraceae, Onagraceae, Poaceae, Polygonaceae, Ranunculaceae and Rosaceae families. In addition, the studied honey samples showed Fabaceae is the most preferred family and *Onobrychis* sp. is the most preferred genus by the bees. Similarly, different studies conducted on Anzer honey revealed that the families most preferred by bees are Apiaceae, Asteraceae, Brassicaceae, Campanulaceae, Fabaceae, Lamiaceae, Liliaceae and Rosaceae (Sorkun and İnceoğlu, 1984; Sorkun et al., 1989; Sorkun and Doğan, 1995; Ulusoy, 2010; Hotaman, 2015). The diversity of Anzer honey is due to the rich flower flora of the region, which has more than 500 plants (of them, 80 are endemic), at altitude of 2300 meters. In addition, the change of this flora in bi-weekly periods is another factor that increases the quality of honey (Sorkun and İnceoğlu, 1984; Sorkun et al., 1989). Therefore, the plant families preferred by the bees also show high similarity to the Cimil Plateau honey, which is located in the same geography and almost at the same altitude as Anzer. This result displays that the honey produced in Cimil and Anzer Plateaus shows similar characteristics. In addition, the fact that the results of the physico-chemical analysis (moisture, sugar, proline) in this study comply with the Turkish Food Codex reveals that Cimil honey can be another flower honey with remarkable features. In sample number 6, *Castanea sativa* pollens were determined dominant by a count of %70. The altitude of this sample is not compatible with the elevation range of natural chestnut habitats. This outcome may be a result of placing plants that give honey superior properties placed around hives, or hives transported to chestnut-populated areas.

Demir (2011), analyzed pollens in 10 honey samples from Komati (Çamlıhemşin) region. Her results show that the taxa of *Castanea sativa*, Ericaceae, Fabaceae and Rosaceae are dominant groups, one honey sample is the unfloral group, and nine honey samples are multifloral groups. Demir (2013) researched 41 honey samples from Ayder-Çeymakçur. By this study, mostly determined the pollen of taxa belong to the Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Ericaceae, Fabaceae, Fagaceae, Gentianaceae, Geraniaceae, Lamiaceae, Lauraceae, Malvaceae, Polygonaceae, Primulaceae, Ranunculaceae, Rosaceae, Salicaceae and Scrophulariaceae families. Malkoç et al. (2019), studied Anzer

honey on the basis of total phenolic composition, melissopalynological analysis and antioxidant properties. By this research, the pollen of the taxa belong to the Apiaceae, Asteraceae, Ericaceae, Fabaceae, Lamiaceae, Liliaceae, and Rocaceae families. Similarly, Taşkın and İnce (2009) investigated pollen in 20 honey samples in the Burdur region. They reported that the taxa of Apiaceae, *Pimpinella anisum* L., *Anthriscus* Persoon, *Cardamine* L., Compositae, *Centaurea* L., Ericaceae and *Dianthus* L. were in dominant groups. The same findings were obtained in honey analyses made in Kars and Giresun regions (Çelemli et al., 2018; Temizer et al., 2016). These palynological findings are similar with our results and also will be helpful for characterizing of Cimil honey.

Altitude is important in terms of pollen content of honey. In the study of Tosunoğlu et al. (2022), pollen diversity was found to be lower in honey at low altitudes and higher pollen diversity was found in honey at high altitudes. Similarly, Kurtagic et al. (2016), investigated the pollen content of honey at different altitude in Bosnia and Herzegovina regions. The researchers stated that the lowest number of pollen grains was found in samples taken from sub-mountain climate regions and the highest pollen grain content was found in samples taken from moderately terrestrial areas (Kurtagic et al., 2016). In our study, it was determined that the pollen content changed significantly according to the difference in altitude. In addition, botanical origin and geographical structure are important for manufacturers and consumers in the classification of honey. With the data we have obtained, it is aimed to increase the market value of Cimil Plateau honey and to contribute to the economy of the community.

The moisture content of honey is crucial for granulation, and high moisture content causes both microbial degradation and crystallization (Tosi et al., 2002). The amount of water varies according to the geographical characteristics of the region where honey is collected, as well as the harvest time and form (Bogdanov et al., 2004). According to the Turkish food codex, the moisture content in flower and secretion honey should be a maximum of 20%. In the present study, Cimil honey, which is flower honey, was found to be in compliance with the Turkish Food Codex, with a moisture content of 18%. According to Demir (2013), the moisture content of honey produced in the Ayder and Çeymakçur Plateaus varies between 16.4% and 20.9%. In addition, a study in which some bioactive properties of Anzer honey and pollen were examined in vitro stated that the moisture rate of worldwide-known Anzer honey varies between 17.90 and 20.27% (Hotaman, 2015). Similarly, Çelemli et al. (2021) found the moisture content of honey produced in the Ayder Plateaus between 15.8% and 18.8%. According to Derebaşı et al. (2014), the amount of moisture in Black Sea region-Türkiye and Rize honey was between 16.6±0.12%-18.57±0.72%, respectively. The data obtained from the present study show that the moisture content of Cimil honey is similar to other honey samples studied in the region and has a similar honey quality to other honey produced in the region.

The proline assay is based on the formation of a colored complex between proline and ninhydrin and is an indicator of the quality of honey as it is the only amino acid added to honey by the bee (Hotaman, 2015). The proline value of Cimil Plateau honey in this study was calculated as between 568.88 and 758.51 mg/kg. According to Hotaman (2015), the amount of proline in Anzer honey was between 808-1139 mg/kg. Bayram and Demir (2018) reported some monofloral and multifloral honey proline content as between 503.46-696.09. This content is close to the proline value of heather (Ericaceae) and Anzer honey given in the literature. In addition, according to the Turkish Food Codex Honey Communiqué, the amount of proline in honey should be at least 300 mg/kg. Thus, the amount of proline in this study is compatible with the reference studies. The amount of proline is a crucial criterion for assessing the protein content of honey. The results of this study indicate that honey may have a high protein content, and the quality may be superior 95% of the dry matter of honey consisted of

carbohydrates. These include monosaccharides (glucose and fructose), as well as disaccharides and high sugars. Most of these sugars are not present in the nectar but are formed by the action of enzymes and acids during the maturation and storage of honey (Ulusoy, 2010).

According to the Turkish Food Codex Honey Communiqué (2020/7), the amount of Fructose + Glucose in the honey must be at least 60 g per 100 g. In the honey samples examined in this current study, the fructose amount was found between 35.36% and 51.44%, and the glucose was between 21.35% and 45.7%. Likewise, Batu et al. (2013) stated that while the Fructose rate was 40.21%, the glucose amount was 33.36% in the flower honey produced in Rize. In addition, the mentioned study also determined that the amount of inverted sugar was 71.01%. In parallel to the studies given above, the fructose and glucose ratios in the honey used in the present study are within the standard reference range. One of the most abundant enzymes in honey is diastase (amylase), and some of the enzymes such as invertase, catalase, glucose oxidase, and phosphatases are secreted into honey from the stomach of the bees (Ulusoy, 2010). In the present study, the number of diastases was found between 9.01-53.95. Batu et al. (2013) revealed that the number of diastases was 17.9 in the honey produced in Rize province. According to the Turkish Food Codex Honey Communiqué and European Union standards, the amount of diastase should be a minimum of 8. The standard diastase value detected in Cimil honey could be an important quality indicator.

One of the important biochemical criteria used in honey quality assessment is HMF analysis (Ulusoy, 2010). A high HMF value indicates overheated honey. HMF occurs as a result of incorrect storage and heat treatment and has negative effects on bee and human health. Due to its harmful carcinogenic effects, high HMF values are restricted (Anonymous, 2020). In the present study, the HMF amount was determined between 0.24 and 2.48 mg/kg. Turkish Food Codex (2020) states that the amount of HMF in high-quality honey should not be more than 40 mg/kg. Equivalently, in the study on honey produced in Rize, the HMF measure was determined to be 4.04 mg/kg (Batu et al., 2013). Similarly, in the investigation on honey produced in Ayder Plateau (Rize), the HMF measure was found in 0,7-11,31 ppm (Çelemli et al., 2021). As a result, the results of HMF analysis in Cimil honey are compatible with the general literature.

Honey and pollen are natural products with a high antioxidant capacity. The geographical features of the region, flower flora, and production techniques are the leading factors that affect these numbers. Especially among local people, the honey produced in the Anzer region is known for its healing effects. The North-Anatolian Anzer region is world-famous for its honey. It is believed that Anzer honey has more health benefits than other honey produced in different regions. Therefore, the price of this special honey is higher. Honey producers and consumers state that Anzer honey is therapeutic for liver, stomach, and intestinal diseases, as well as some skin conditions. Some studies state that the healing property of this honey is related to the floral richness of the Anzer plateau, which contains about 80 endemic species, and has more than 500 different flowers (Ulusoy, 2010; Hotaman, 2015). Cimil Plateau is in the same geographical area as Anzer. However, the flora of this area has not been studied, and various quality assessments have not been conducted on honey produced in the region. These situations have limiting effects on the recognition of Cimil honey. In so much that, commodities of Cimil honey producers are sold at prices below their value. The data obtained with this study reveal that important criteria for honey quality, such as pollen variety, moisture, and proline amounts, are quite similar for Anzer and Cimil honey. In addition, these results obtained for the physicochemical properties of Cimil Plateau honey show that it has a good quality level, adequate processing, good maturity, and freshness.

5. Conclusion

Melissopalynological analysis is important for the characterization of honey samples according to

their plant origins. In addition, this analysis provides important data on the plant diversity of the location where honey is collected. As a result of the melissopalynological analysis of honey samples from Cimil plateau in İkizdere district of Rize province, 8 genera and 3 taxon belonging to 18 families were determined.

Melissopalynologic studies showed that the pollens dominantly belong to *Castanea sativa* (Fagaceae) taxon and *Onobrychis* sp. (Fabaceae) genera. Secondary pollens are defined to belong to 8 Campanulaceae, Ericaceae, Fabaceae, Lamiceae, Onagraceae, Ranunculaceae, Rosaceae, and Tiliaceae families. The presence of pollen belonging to more than one family in honey samples shows that the honey is of high quality and rich in nutrients.

Cimil Plateau is a favorable location for beekeeping. Its flora comprises various plant species that have nectar potential for honeybees. Owing to the pollen analysis of honey samples from Cimil Plateau, the plant variation of the region was reflected.

The moisture content of the samples was determined to be 16.9-19.1 %, and the proline content was determined to be 568-758 mg/kg. In sugar analysis, it was determined that Fructose was 35.36-51.4 g/100 g and Glucose was 21.35-65.78 g/100 g. This is the first report performed on Cimil honey based on melissopalynological and physico-chemical characteristics. It has been observed that the melissopalynological and physico-chemical analysis results of honey are consistent with the European Union Standards and Turkish Food Codex Honey Communiqué (2020/7).

Acknowledgements

This study is financially supported by TUBITAK 2209 A (Project number: 1919B012003596).

Author Contributions

G. Yetkin: Resources; **S. Kundakçı:** Investigation, Writing – Review & Editing; **E. Demir:** Methodology; **K. Coşkunçelebi:** Data Curation, Formal Analysis, Roles/Writing-Original Draft; **S. Makbul:** Project Administration, Visualization

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