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Research Article

Investigation of Some Quality Criteria in Chestnut Honey of Düzce Province¹

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ABSTRACT

Honey is a natural food source that has been consumed by humans for thousands of years and has an important place in various cultures. Chestnut honey is an important type of honey produced in Düzce province. Determining the quality and purity of honey ensures that consumers have access to safe, high-quality honey. The control criteria for the suitability of honey for human health consumption are defined in the Turkish Food Codex Communiqué on Honey (2020/7). Düzce University Scientific and Technological Research Application and Research Center (DÜBİT) responds to requests for honey analysis for non-commercial, internal quality control purposes. In this study, the results of honey samples produced in the province of Düzce and analyzed in the laboratories of DÜBİT were examined according to the criteria of the Turkish Food Codex Communiqué on Honey and an evaluation of the results was carried out. According to the test results, 38%, 20% and 16% of the samples were found to be unsuitable for the quality criteria, especially for the sugar profile, conductivity and proline parameters, respectively. Additionally, pollen analysis of 13% of the honey samples revealed pollen densities that did not match the declared plant family. From a public health point of view, it is important to consume high-quality, reliable and natural honey products. Therefore, quality control of bee products should be carried out from the producer to the consumer.

Keywords: chestnut honey, natural product, honey analyses, bee product, quality criteria

Düzce İli Kestane Balında Bazı Kalite Kriterlerinin Araştırılması

Öz

Bal, binlerce yıldır insanlar tarafından tüketilen ve çeşitli kültürlerde önemli bir yere sahip olan doğal bir besin kaynağıdır. Kestane balı Düzce ilinde üretilen önemli bir bal türüdür. Balın kalitesinin ve saflığının belirlenmesi, tüketicilerin güvenli, yüksek kaliteli bala erişimini sağlar. Balın insan sağlığı açısından tüketime uygunluğuna ilişkin kontrol kriterleri Türk Gıda Kodeksi Bal Tebliği'nde (2020/7) tanımlanmıştır. Düzce Üniversitesi Bilimsel ve Teknolojik Araştırmalar Uygulama ve Araştırma Merkezi (DÜBİT), ticari olmayan, iç kalite kontrol amaçlı bal analizi taleplerine yanıt vermektedir. Bu çalışmada Düzce ilinde üretilen ve DÜBİT laboratuvarlarında analiz edilen bal numunelerinin sonuçları Türk Gıda Kodeksi Bal Tebliği kriterlerine göre incelenmiş ve sonuçların bir değerlendirmesi yapılmıştır. Test sonuçlarına göre örneklerin %38'i şeker profili, %20'si iletkenlik ve %16'sı prolin

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parametreleri başta olmak üzere kalite kriterlerine uygun bulunmamıştır. Buna ek olarak, balların %13'ünde yapılan polen analizinde, beyan edilen bitki familyasına uymayan polen yoğunlukları tespit edilmiştir. Halk sağlığı açısından bakıldığında, yüksek kaliteli, güvenilir ve doğal bal ürünlerinin tüketilmesi önemlidir. Bu nedenle arı ürünlerinin kalite kontrolü üreticiden tüketiciye kadar yapılmalıdır.

Anahtar Kelimeler: kestane balı, doğal ürün, bal analizleri, arı ürünü, kalite kriterleri

I. INTRODUCTION

The latest legislation concerning honey is the Turkish Food Codex Honey Communiqué (Communiqué No: 2020/7) which was published in 2020. Honey is defined in this Communiqué as a natural substance that can naturally crystallize. It is created when honey bees collect plant nectars, secretions from living plant components, or secretions from plant-sucking insects living on the living components of plants and then modify them by combining them with exclusive substances, reducing the water content, and maturing by storing them in the honeycomb. The earliest discoveries of honey production date back to 7000-8000 BC. Archaeological evidence suggests that as hunter-gatherer societies adopted a sedentary lifestyle, they began developing tools and containers specifically for honey production [1]. Honey holds significant importance for humanity with regard to its historical significance. Its journey can be traced back to the ancient civilizations of Egypt, Greece and Mesopotamia, and continues to be relevant today. The industrial revolution, advancements in honey production and beekeeping, and the utilization of innovative technology and techniques have led to the modern form of beekeeping [2].

Honey can be classified into two types based on its plant source: secretion honey and flower honey. Worker bees collect nectar from flowers to produce flower honey, while secretion honey is made by bees collecting resin and sweet secretions from insects living in forest trees. Honey can also be categorized based on the type of flowers bees visit while collecting nectar: polyfloral (multi-flowered) honey and monofloral (single-flowered) honey. The most well-known varieties of monofloral honeys are lavender honey, chestnut honey, carob honey, orange blossom honey, and lemon blossom honey [3]. Differences in the plant source significantly affect the smell, taste and color of honey. Other physical properties and chemical content of honey also vary according to the type of bee, the climatic characteristics of the geographical region where it is collected and the harvest season. All these factors contribute to determining the quality of honey. Multi-flowered honeys are produced in high altitude plateaus and depending on the flower diversity of the region [4], [5].

Turkey plays a significant role in honey production. With its unique geographical features, rich flora housing endemic plant species, and diverse honeybee breeds, Turkey has high potential for beekeeping and producing high-quality honey [6]. Chestnut honey matures as it is derived from collecting nectar from chestnut trees that start to bloom at the start of the summer season. The use of chestnut as a raw material is notable due to its chemical and biological benefits in traditional medicine. In Turkey, *Castanea sativa* Miller (European chestnut) produces a monofloral variety of honey when chestnut trees in the Marmara, Aegean, and Black Sea regions flower in June [7], [8].

Monofloral chestnut honey is abundant in C and B vitamins, along with iron and potassium minerals. Its dark color distinguishes chestnut honey from other honey varieties. Studies have shown that chestnut honey improves immunity through its vitamin, mineral, and antioxidant content. Additionally, it possesses antiviral and antibacterial properties [9].

In conclusion, honey and chestnut honey hold significant economic and cultural significance in Turkey. They are widely consumed within the country and exported abroad. Additionally, the health benefits of chestnut honey are increasingly recognized. Producers seeking to ascertain the quality of their product and maintain internal control seek out laboratories that conduct honey analyses. DÜBİT, the Scientific

and Technological Research Application and Research Center at Düzce University, conducts honey analyses upon request. The analyses are intended for non-commercial internal quality control purposes and to support local honey producers. The honey samples from the Düzce province, which were analyzed in the DÜBİT laboratory, were evaluated based on the TKG Honey Communiqué criteria in this study.

II. MATERIAL AND METHODS

A. MATERIAL

The study discusses the conformity of the analyses conducted by Düzce University Scientific and Technological Research Application and Research Centre (DÜBİT) using the honey analysis methods determined by the Turkish Standards Institute with the Turkish Food Codex Honey Communiqué. Only data of customers who authorized its use were utilized. Technical abbreviations were explained when first used. The analyses performed in DÜBİT between 2020-2023 served as the database.

B. METHODS

Turkish Standard methods and certain International Honey Commission (IHC) methods were utilized to perform analyses specified in the Turkish Food Codex Honey Communiqué [10], [11].

B. 1. Determination of Moisture Content

The lens of the digital honey refractometer was initially calibrated by introducing pure water. Afterwards, a small quantity of honey sample was inserted, and the moisture level was measured. This sequence was repeated thrice. The water content of honey was quantified as the mass of water per 100 g of honey. The refractive index of liquidized honey was calculated [5], [12].

B. 2. Determination of Diastasis Number

The diastase activity of honey samples is conducted in accordance with TS 13364 guidelines. The amylase enzymes present in 100 grams of honey facilitate starch breakdown at a specific temperature range (38 to 40 degrees Celsius) and under predetermined experimental conditions. This method measures the enzyme activity in 1 gram of honey, ascertained by observing the blue value at the endpoint, after exposing it to 0.01 grams of starch at a temperature of 40 degrees Celsius for an hour [13], [14].

B. 3. Measurement of Electrical Conductivity

The experiment was conducted in accordance with TS 13366 standards. Subsequently, 20 g of dry honey was weighed and dissolved in 100 ml of distilled water using the method outlined in TS 13365. The electrical conductivity of the mixture was measured with a conductivity cell to determine its resistance [15], [16].

B. 4. Determination of Free Acidity

The free acidity of the honey samples was determined according to the guidelines outlined in TS 13360. Specifically, the free acid content of the honey sample was dissolved in water and was measured through potentiometric titration with the use of sodium bicarbonate solution until the endpoint was at pH 8.3. Results were then expressed as mmol/kg in sodium hydroxide for every 100 g of honey using the method specified in this standard [13], [17].

B. 5. Determination of Proline Content

Proline assays follow harmonized methods as specified by the IHC and serve as crucial parameters in detecting honey adulteration. Proline content represents a quality indicator, forming a colored complex with ninhydrin. Detection of this complex involves the addition of 2-propanol, followed by observation for color change at the maximum wavelength of the sample solution and the reference solution [10], [12].

B. 6. Determination of Sugar Profile

Fructose, glucose, sucrose, and maltose were identified in honey through sugar profile analysis, which utilized IHC. High-performance liquid chromatography (HPLC) was employed for the analyses. To prepare, 5 grams of honey was dissolved in 40 Milliliters of ultrapure water and the solution was filled up to 100 ml with ultrapure water. The filtered and vialled sample was subsequently analyzed using HPLC. Acetonitrile: water (80:20) was utilized as the mobile phase for the experiment. In addition, a column of NH₂ (5 µm, 250 mm x 4.6 mm, 100 Å) was selected as the desired separation column [5], [10], [13], [18].

B. 7. Determination of Hydroxymethylfurfural

HMF analysis was carried out according to the IHC-determined method. The HMF value was measured in the samples using HPLC with a C-18 column. The measurements were compared to HMF standards that were measured at 258 nm [10], [13].

B. 8. Pollen Analysis

Relative pollen analysis was conducted following TS 13363 with minor adjustments. Initially, approximately 10 grams of honey were dissolved in 20 Milliliters of distilled water. The honeycombs were filtered through a 0.5 mm sieve prior to dissolution to eliminate significant solid particles and residual honeycomb materials. The supernatant was discarded after centrifugation at 4,000 rpm for 10 minutes. The pellet is again dissolved with 20 mL of distilled water and centrifuged once more to completely dissolve the sugar crystals. The pellet was then re-dissolved in 200 µl of pure water, and the hydrated pollen was examined under light microscopy at a magnification of 10-40X [19]–[21]. Pollen atlases and literature were utilized to determine the pollen species [22]–[24].

III. RESULTS AND DISCUSSION

We examined some quality criteria in honeys from the Düzce region that were applied to DÜBİT for analysis. We evaluated the conformity of the results we obtained according to the limit values in the annex of the Turkish Food Codex (TFC) Honey Communiqué (Table 1). Depending on the parameter requested, the number of samples analyzed was between 16-30. Although the majority of the samples were found to be suitable according to the criteria of the TFC Honey Communiqué, samples outside the limit values were also detected.

Table 1. Analysis results for chestnut honey from the Düzce and the TFC Honey Communiqué limit values.

Test Parameters	NOS (NUS)	Minimum (min.)	Maximum (max.)	Mean + SD	Limits and Units**
Fructose / Glucose	16 (1)	1,17	1,97	1,40±0,17	1,0-1,85
Fructose + Glucose	16 (6)	34,30	70,22	58,75±9,80	min. 60 g/100g
Sucrose	16 (0)	0,13	3,90	1,37±1,30	max. 5 g / 100g

Maltose	16 (0)	0,65	3,19	1,75±0,83	max. 4 g / 100g
Hydroxymethylfurfural (HMF)	18 (0)	1,10	2,05	1,63±0,40	max. 40 mg/kg
Diastase	18 (2)	7,00	50,00	15,28±11,11	min. 8
Proline	19 (3)	81,60	1202,40	736,80±286,24	min. 500 mg/kg
Conductivity	20 (4)	0.07	1.75	1.00±0.48	min. 0,8 mS/cm
Free acidity	20 (0)	12,00	46,00	27,86±9,63	max. 50 meq/kg
Moisture content	26 (4)	12,50	29,40	18,40±2,86	max. %20
Chestnut pollen ratio	30 (4)	40,00	97,00	80,29±15,44	min. %70

NOS: Number of samples, NUS: Number of unsuitable samples

**Limit values are determined according to the Turkish Food Codex Honey Communiqué.

A low moisture content in honey signifies its maturity and enables its long-term storage without spoilage. Abbreviations for technical terms will be clearly explained upon first use. The water content of honey, also called moisture content, is an important criterion for assessing honey quality. The moisture level of honey differs during the ripening process, depending on environmental conditions, the water content of the nectar that creates the honey, and storage conditions after extraction. High levels of moisture can accelerate crystallization in some varieties of honey, resulting in an increase in water activity that could lead to yeast growth and eventual fermentation. Honey with elevated moisture levels is particularly susceptible to fermentation. In accordance with the TFC Honey Communiqué, the acceptable moisture content for flower honey (excluding puree and heather honey) should not exceed 20%.

Out of the 26 samples of honey that were analyzed in our laboratory, 22 had a moisture content that fell below the limit value of 20%. However, four samples were identified as having values exceeding 20%, with a range of 20.5% to 29.4%. The average moisture content of the analyzed chestnut honey from the Düzce region was determined to be $18.40 \pm 2.86\%$. In 2018, Bayram and Demir found that the moisture content of six chestnut honey samples from the Giresun and Rize regions ranged between 17.8% and 22% [12]. Only one of the tested samples exceeded the limit of 20%, indicating potential issues. The moisture content of chestnut honey from the Kocaeli region was found to be 18.2% in a separate study evaluating quality criteria across 12 types of honey, including chestnut honey [13], which is comparable to our average moisture value. In a previous analysis of honeys from the Düzce region, Kambur *et al.* [25] found moisture levels in two samples dominated by chestnut pollen to be less than 20%, which is consistent with our own findings.

Organic acids such as gluconic acid, acetic acid, butyric acid, citric acid, formic acid, lactic acid, and malic acid can be found in honey. The free acidity of honey represents the total amount of organic acids in it. This parameter is crucial as it affects the taste and quality of honey. When the free acidity level is too high, the honey is considered to be of low quality. The free acidity level in honey may vary depending on the type of honey, production method, and storage conditions. According to the TFC Honey Regulations, the free acidity level for flower honey should not exceed 50 meq/kg. The entire set of 20 samples subjected to the free acidity parameter test revealed results below the prescribed limit. The samples exhibited an average value of free acidity at 27.86 meq/kg, with the lowest and highest recorded levels at 12.5 meq/kg and 46.0 meq/kg, respectively. In 2016, a study examined 10 samples of honey sourced from the Yığılca district of Düzce province. Only one sample did not meet the TFC Honey Regulations criteria for the free acidity parameter, with a value of 70 meq/kg [25].

The electrical conductivity of honey is determined by the quantity of organic acids, proteins, sugars, and minerals present in it. When the electrical conductivity of honey is high, the concentration of these components is also high. Certain types of honey are anticipated to have low conductivity, whereas others

like chestnut honey are expected to have high conductivity. According to the TFC Honey Communiqué, the electrical conductivity of flower honey should not exceed 0.8 mS/cm, whereas that of chestnut honey should be at least 0.8 mS/cm. The electrical conductivity of honey may be contingent upon the type of honey, the mode of production, and storage conditions. For instance, heat-treated honeys exhibit higher conductivity than natural honeys, while temperature or extended storage duration may elevate conductivity. Four out of the 20 samples tested for conductivity failed to meet the minimum chestnut honey limit of 0.8 mS/cm. Upon analyzing all samples, the average conductivity value was found to be 1.00 ± 0.48 mS/cm. The four samples that did not meet the required value had conductivity values ranging from 0.07 to 0.60 mS/cm. In a study where one chestnut honey was compared to twelve other flower honeys, the conductivity value was determined to be 1.97 mS/cm. The study highlights chestnut honey's distinctive conductivity parameter in contrast to other flower honeys [13]. Furthermore, a study analyzing honey samples from the Yığılca region found that one of the two honey samples with dominant chestnut pollen had a conductivity of 0.8 mS/cm, while the other had a conductivity of 0.39 mS/cm [25].

The diastase enzyme is responsible for converting starch to sugar in honey. The diastase level in honey indicates its freshness, with honey high in diastase considered fresh. Additionally, the diastase level provides information about the purity and nutritional value of honey. Adulterated or blended honey has lower diastase levels compared to pure and natural honey. Honey with high diastase levels is considered higher quality than honey with low diastase levels. Enzyme activity in honey analysis reports is calculated based on its potential to convert starch and is expressed as diastase units. According to the TFC Honey Communiqué, this value must be at least 8 for blossom honey. Of the 18 honey samples analyzed, two did not meet the criterion and had a value of 7. The remaining 16 samples had values of 8 or above, with three exhibiting diastase numbers above the average, featuring values of 23, 39, and 50. According to a study conducted in the Düzce region, eight out of ten honey samples examined had diastase numbers exceeding 8, while two lacked diastase [25].

Proline is a fundamental amino acid that constitutes the building blocks of protein. Its significance in honey quality control standards is crucial due to its production solely by honeybees. Proline serves as one of the most relevant indicators that confirm natural and pure honey production by bees. The concentration of proline in honey can fluctuate depending on the honey type, production technique, and storage conditions. Heated honey has lower proline content than traditionally produced honey, and prolonged storage in high-temperature conditions can also decrease its proline levels. According to the TFC Honey Communiqué, chestnut honey must contain a minimum of 500 mg/kg of proline. Of the nineteen honey samples analyzed in this study, sixteen had proline levels above 500 mg/kg (with a maximum of 1202.4 mg/kg), while three samples showed proline levels below the limit value (minimum 81.6 mg/kg). In a study examining various flower honeys from the Erzurum, Giresun, Hakkari, and Rize regions, Giresun and Rize honeys were identified as chestnut honey through palynological evaluation. The study reported proline levels between 503.46-692.88 mg/kg, highlighting the low proline content in samples determined as chestnut honey [12]. In a recent study conducted by Özgüven *et al.* [13], the quantity of proline present in chestnut honey sourced from the Kocaeli region was determined to be 711 mg/kg.

Hydroxymethylfurfural (HMF) is a compound naturally present in honey and molasses. HMF forms through the enzymatic degradation of fructose in honey under high temperatures or during extended storage periods. The HMF concentration in honey serves as a measure of its quality. According to the TFC Honey Communiqué, the acceptable HMF level in honey should not exceed 40 mg/kg. Furthermore, the HMF level may increase due to the storage environment, particularly at high temperatures or prolonged storage. None of the 18 samples analyzed by the HPLC method had a value of 40 mg/kg or higher in terms of HMF content. Only 3 samples were found to contain HMF, with the highest HMF value recorded at 2.05 mg/kg. In their study, Özgüven *et al.* [13] found HMF content in chestnut honey to be less than 0.1 mg/kg, and in other floral honeys, values were found to be up to 1.22 mg/kg. Kambur *et al.* [25] found that the HMF values of 10 types of honey examined were within the limit values. However, the results (ranging from 17.93-36.02 mg/kg) were considerably higher than the literature values.

Approximately 80% of honey's contents are sugars. The main sugars found in honey are fructose and glucose, but sucrose, maltose, galactose, and xylose can also be present. Honey's sugar profile provides detailed information on quality, purity, and adulteration. Sugar quantities in honey can be affected by the type of honey, production method, heat treatment, and storage conditions. The TFC Honey Communiqué outlines four parameters and limit values for sugar content in flower honey. These parameters include the ratio of fructose to glucose (between 0.9-1.4; for chestnut honey, between 1.0-1.85), the total amount of fructose and glucose (minimum of 60 g per 100 g), maximum amount of sucrose (5 g per 100 g), and maximum percentage of maltose (4%). One of the 16 honey samples analyzed using the HPLC has a fructose/glucose ratio of 1.98, with fructose and glucose values of 41.14 g/100g and 20.8 g/100g, respectively, falling outside the limit range. Six samples with fructose and glucose values ranging from 34.3 g/100g to 57.45 g/100g, below the minimum expected total of 60 g/100g, also failed to meet the requirement. All samples were evaluated as suitable with respect to the limit values of sucrose and maltose sugars. The detection and separation of sugars and HMF in the HPLC chromatogram can be seen in Figure 1.

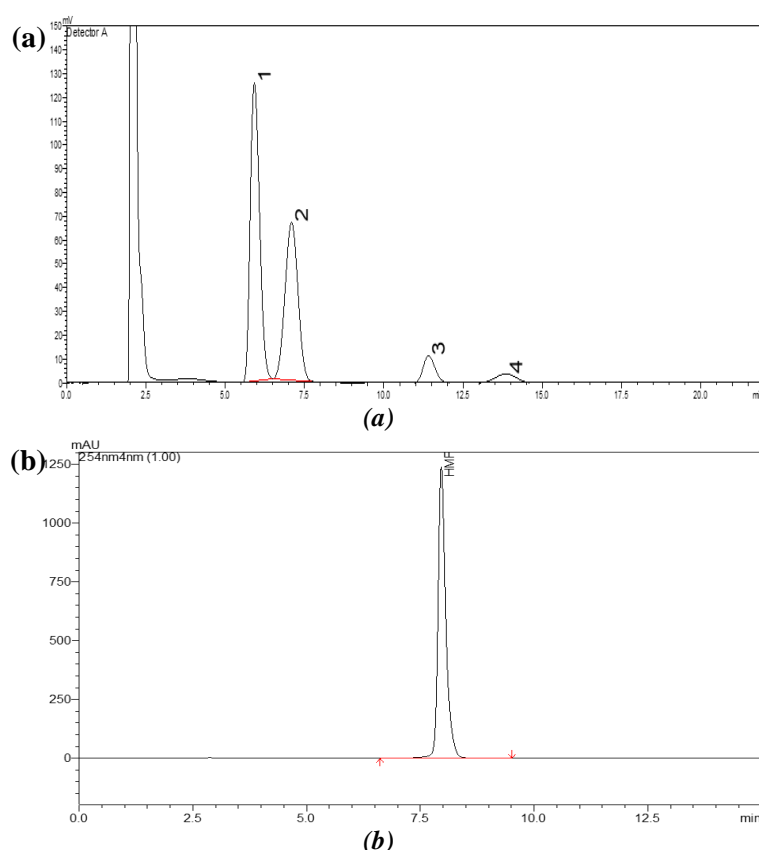


Figure 1. (a) Sugars and (b) HMF – chromatograms of honey HPLC analyses. The numerical digits in Figure (a) indicate 1: fructose, 2: glucose, 3: sucrose, and 4: maltose.

When analyzing recent studies, comparable outcomes are noticed. Ten honey samples from the surroundings of Yığılca were analyzed, indicating fructose/glucose ratios ranging from 1.18 to 1.32 within the limit values. Furthermore, nine of these samples were found to contain maltose in amounts lower than 1.25 g/100 g [25]. In a study analyzing chestnut and rhododendron honey samples obtained from regions including Bartın, Kastamonu, İstanbul, and Düzce (6 samples), the results of sugar analysis by HPLC for 18 samples indicate fructose/glucose ratios ranging from 1.17 to 1.80. In terms of the sum of fructose and glucose parameters, half of the tested samples did not reach the 60g/100g limit value [18]. In another study conducted on flower honeys, the ratio of fructose/glucose in chestnut honey was recorded as 1.49 and the total amount of the sum of fructose and glucose was 78.58. In addition, the sucrose sugar amount was also determined as 0.44 g/100g [13].

Relative pollen determination in honey is a technique utilized to identify the plant-based pollen present in honey. Melissopalynology, the scientific study of pollen and spores in honey, utilizes the melissopalynological approach to ascertain the amount, family, taxon or species of pollen grains found in honey. According to the TFC Honey Communiqué (2020/7) Annex-2, the minimum pollen content for honeys whose botanical source is declared is specified. Chestnut honey must contain at least 70% *Castanea sativa* pollen to be declared as such. After conducting a melissopalynological analysis of 30 honey samples submitted to our laboratory as chestnut honey, it was discovered that six of these samples did not meet the threshold of 70% in regard to Fagaceae and *C. sativa* pollen content (Figure 2). While chestnut pollen is dominant in all other honeys, secondary or minor levels of pollen belonging to other families such as Fabaceae, Ericaceae, and Tiliaceae, which are part of the dominant vegetation in the area, were detected (Figure 2).

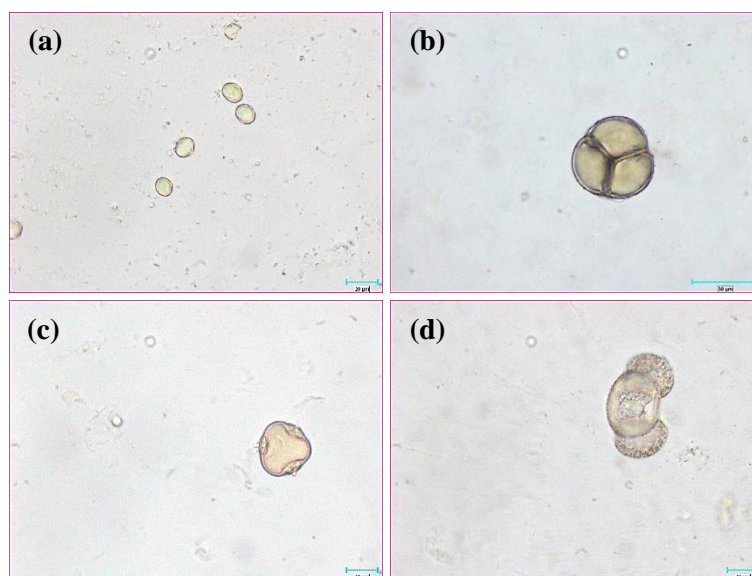


Figure 2. (a) Fagaceae, (b) Ericaceae, (c) Tiliaceae, and (d) Pinaceae – Examples of microscopic photographs of hydrated pollen grains from different plant families.

Upon examining prior studies on Düzce region honeys, comparable outcomes were observed to our own findings. Kambur *et al.* [25] identified three monofloral honeys through the analysis of honey samples from the Yığılca region. Two of them were chestnut honeys (with pollen ratios of 48.2% and 45.75%, respectively), while the other was honeysuckle honey (with a pollen ratio of 46.2%). In another study that included six samples from Düzce and examined the palynology of honey from four cities, it was reported that out of 18 samples, 10 were monofloral chestnut. Four of the honeys from Düzce have been identified as monofloral chestnut honey, with pollen ratios ranging from 48% to 68% [18]. In another study examining the honey from the Düzce Yığılca region, pollen detection of 43 different taxa from 26 families was conducted. In two of the seven samples, *C. sativa* pollen was recorded dominantly with percentages of 91.5% and 92.5% [20]. Another palynological study conducted in the Yığılca region examined chestnut honey propolis, and identified *C. sativa* pollen at a rate of 80.5% [26]. A separate study analyzed 34 honey samples collected from Düzce province and districts in 2019 and 2020, detecting pollen from 109 taxa belonging to 20 different families. *C. sativa* had the highest pollen rate among all identified taxa. It has been reported that 8 out of 12 monofloral honey samples collected were chestnut honey, with a pollen ratio of 70% or higher [19]. Similar to many studies, the prevalent pollen species identified in honey from the Düzce region are associated with the vegetation in the area, namely Fagaceae and Ericaceae families. Furthermore, pollen from numerous other families, including Fabaceae, Apiaceae, Malvaceae, Asteraceae, Boraginaceae, Poaceae, Rosaceae, and Campanulaceae, were also detected but classified as secondary or minor.

IV. CONCLUSION

In this study, we evaluated the quality criteria of chestnut honey from the Düzce region, which were analyzed at DÜBİT laboratories. The TFC Honey Communiqué was used to ensure conformity. While most samples met the quality criteria, some exhibited inappropriate values.

Honey is a fundamental foodstuff in human life, with the quality of honey being critical when assessing its nutritional value. Although society has yet to fully comprehend the significance of determining and regulating quality criteria when purchasing honey, it plays a vital role in ensuring access to high-quality, natural honey. Information campaigns should be initiated by local and general authorities to raise public awareness. These campaigns should encourage producers to test and analyze their products, while also urging consumers to be mindful when making purchases. It is more accurate to evaluate honey using quality criteria data rather than relying on the belief that every expensive product is of good quality, and every cheap product is of poor quality. This approach would protect both parties involved.

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