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## Melissopalynological and physicochemical properties of chestnut honey produced in Zonguldak, Türkiye

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### Abstract

This study aimed to determine the melissopalynological and physicochemical properties of chestnut honey harvested in Zonguldak Province during the 2025 production season. For this purpose, nine chestnut honey samples were utilized. Physicochemical parameters were determined by refractometry and High-Performance Liquid Chromatography (HPLC), while the botanical origin of the honey samples was verified by melissopalynological analysis under light microscopy. Melissopalynological analysis showed that *Castanea sativa* was the dominant pollen type, accounting for an average of 82.28%, which substantiates the unifloral character of the honey samples. Physicochemical analyses showed that the honey samples had an average moisture of  $16.82 \pm 1.42\%$ , electrical conductivity of  $1.32 \pm 0.20$  mS/cm, fructose content of  $36.71 \pm 3.21\%$ , glucose content of  $29.32 \pm 2.64\%$ , total fructose + glucose content of  $66.03 \pm 5.21\%$ , and a fructose/glucose ratio of  $1.26 \pm 0.10$ . In addition, the HMF content was  $2.36 \pm 2.67$  mg/kg, diastase activity was  $29.48 \pm 8.63$  DN, and proline was  $877.74 \pm 189.58$  mg/kg. All analyzed samples complied with the limits specified in the Turkish Food Codex Honey Communique and exhibited characteristics comparable to those of high-quality chestnut honeys reported in the literature. These results contribute to the literature on the physicochemical and melissopalynological characterization of chestnut honeys produced in Zonguldak and provide data for quality assessment.

**Keywords:** Chestnut honey, Honey, Honey quality, Melissopalynology, Zonguldak

### INTRODUCTION

Honey is a natural product that serves as the primary energy source for bees, formed by collecting plant nectar or botanical secretions and maturing them within honeycomb cells. Based on the nectar source, honey is classified into two categories: blossom honey and honeydew honey. While blossom honey is derived from floral nectar secretions of plants, honeydew honey originates from botanical substances or excretions of insects living on plants (Sorucu 2019, Apan et al. 2021). Honey obtained predominantly from a single floral species is termed unifloral; chestnut honey serves as a prominent example of such unifloral honeys (Chirsanova et al. 2021).

The color of chestnut honey varies from brown to black. Its flavor, which fluctuates depending on the chestnut pollen concentration, is characterized by a slightly bitter taste, unique aroma, and subtle tannic structure (Pehlivan 2023). Due to its high fructose content, it exhibits resistance to crystallization,

thereby ensuring a long shelf life (Yang et al. 2012). Approximately 95% of the dry matter of honey consists of sugars. The sugar fraction of honey is primarily composed of fructose and glucose, and the fructose + glucose (%) (F+G) content, along with the fructose/glucose ratio (F/G), are important parameters for evaluating honey quality and degree of maturation. In addition, honey contains various disaccharides, such as sucrose, maltose, turanose, and isomaltose, as well as oligosaccharides such as melezitose, erlose, and raffinose, in varying concentrations (Da Silva et al. 2016). Honey also contains small amounts of proteins, amino acids, enzymes, vitamin C, B-complex vitamins, and minerals such as iron, zinc, magnesium, and copper. Furthermore, honey has a rich composition of antioxidant compounds, particularly flavonoids and phenolic acids (Bogdanov et al. 2008, Barreira et al. 2010, Simsek et al. 2021).

The physicochemical characteristics of honey significantly influence its quality. Moisture, ash content, electrical conductivity, free acidity, hydroxymethylfurfural (HMF) content, and diastase activity are the fundamental quality criteria (Bogdanov et al. 1999). Excessive moisture is a major quality defect that destabilizes honey and promotes fermentation. Such elevated levels are commonly observed in honeys harvested before full maturation (Singh and Singh 2018). The electrical conductivity of honey is associated with its mineral and acid content and serves as a critical parameter for determining botanical origin. An increase in free acidity indicates that the honey has undergone fermentation (Toy and Şahinler 2022).

HMF levels and diastase activity are indicators of freshness and processing history; HMF increases, and diastase activity is altered in honeys that are improperly stored, overheated, or adulterated with sugar (Tosi et al. 2002, Sak-Bosnar and Sakač 2012). Proline is the primary amino acid found in honey, and the International Honey Commission (IHC) recognizes proline as an indicator of honey maturity and potential sugar adulteration (Sedláčková et al. 2021). Although chestnut honey is produced in many countries worldwide, its production is heavily concentrated in Türkiye, particularly in the Black Sea, Marmara, and northern Aegean regions (Taş-Küçükaydın et al. 2023).

Zonguldak is situated in the western Black Sea region, where chestnut trees grow naturally, leading to widespread chestnut-honey production. This study aimed to characterize the melissopalynological and physicochemical properties of chestnut honeys produced in Zonguldak, evaluate their quality in accordance with the Turkish Food Codex Honey Communique (TFC-HC), and compare the results with previously reported chestnut honeys from different regions.

## MATERIALS AND METHODS

### Chestnut Honey Samples

Chestnut honey samples were selected from samples brought to the Zonguldak Beekeepers Association (ZAYBİR) facility by its members for bottling and packaging during the 2025 production season. A total of nine chestnut honey samples produced within the borders of Zonguldak province were included in this study (Figure-1) and coded as Z1–Z9. Laboratory analyses were performed at the Kastamonu University Central Research Laboratory Application and Research Center.

### Melissopalynological Analysis

Melissopalynological analyses were performed to characterize the botanical origin of honey samples

according to the methods described by Sorkun (2008) and Louveaux et al. (1978). For this purpose, 10 g of honey was mixed with distilled water and a *Lycopodium spp.* tablet, stained with basic fuchsin, and centrifuged. The prepared slides were examined under a light microscope, and pollen grains were identified microscopically according to Wodehouse (1935). The proportional distributions (%) of pollen types were calculated, and the botanical origins of the honey were determined.



**Figure 1. Location of Zonguldak province in the western Black Sea region of Türkiye.**

### Physicochemical Analyses

The moisture, electrical conductivity, sugar profile (fructose, glucose, F+G, F/G ratio, sucrose, and maltose), HMF, diastase activity, and proline of the honey samples were determined. The analyses were performed based on the methodologies reported by Uzunca et al. (2023). All analyses were performed in triplicate. In this context, moisture was determined by refractometry. The sugar profile and HMF levels were analyzed by High-Performance Liquid Chromatography (HPLC) according to IHC-recommended methods. For each parameter, data were expressed as mean, standard deviation, and minimum-maximum values. The results were evaluated based on the TFC-HC (Communique No. 2020/7).

### Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics 20 software. The normality of the data distribution was evaluated using the Shapiro–Wilk test. To evaluate the impact of pollen content on the physicochemical properties, honey samples were classified into two groups based on their *Castanea sativa* pollen levels: low pollen (70–80%) and high pollen (>80%). Differences between groups were analyzed using one-way analysis of variance (ANOVA). Tukey's post hoc test was used to identify specific differences in cases of significant

differences ( $p < 0.05$ ). A one-sample t-test was applied to evaluate whether the physicochemical parameters of the samples complied with the limits specified in the TFC-HC. Pearson correlation analysis was conducted for normally distributed data, with significance levels set at  $p < 0.05$  and  $p < 0.01$ .

## RESULTS

The results of the melissopalynological analyses are presented in Table-1. The analysis revealed that *Castanea sativa* pollen was predominant, with an average representation of  $82.28 \pm 5.60\%$ . Furthermore, pollen grains belonging to the families Fabaceae, Cistaceae, Rosaceae, Ericaceae (*Rhododendron sp.*), Brassicaceae, Malvaceae, Cornaceae, Adoxaceae, Asteraceae, Ranunculaceae, Tamaricaceae, and Apiaceae were identified as secondary pollen sources. The results of the physicochemical analyses are presented in Table-2. The mean moisture of the samples was  $16.82 \pm 1.42\%$ , with values ranging from 15% to 19.03%. The electrical conductivity averaged  $1.32 \pm 0.20$  mS/cm, with a range of 1.06-1.63 mS/cm. Regarding sugar composition, the mean percentages of fructose and glucose were  $36.71 \pm 3.21\%$  and  $29.32 \pm 2.64\%$ , respectively, with a total of  $66.03 \pm 5.21\%$ . The F/G ratio was calculated as  $1.26 \pm 0.10$ , ranging from 1.15 to 1.42.

The average maltose content was  $2.80 \pm 0.66\%$ . The mean HMF concentration was  $2.36 \pm 2.67$  mg/kg, with a maximum of 7.2 mg/kg. HMF was not detected in samples Z4, Z6, and Z9. In terms of enzymatic

activity, the mean diastase activity was  $29.48 \pm 8.63$  DN, ranging from 18.2 to 38.9 DN. Finally, the proline, an essential indicator of honey maturity and freshness, averaged  $877.74 \pm 189.58$  mg/kg, with minimum and maximum values of 647 mg/kg and 1172 mg/kg, respectively. Sucrose was detected only in sample Z8 at 0.16%, well below the 5% limit established by the official communique (Table-3).

The samples were categorized into two groups (low pollen: 70–80% and high pollen: >80%) to determine the effect of *Castanea sativa* pollen density on physicochemical properties. According to the one-way ANOVA results, no statistically significant differences were observed between the two groups for any of the tested parameters, including moisture, electrical conductivity, sugar profile, diastase activity, and proline content ( $p > 0.05$ ). According to the results of the one-sample t-test applied to the honey samples, moisture, HMF, and sugar profile were significantly below the legal maximum limits, whereas quality parameters such as proline and diastase activity were significantly above the legal minimum limits ( $p < 0.01$ ).

Correlation analysis revealed that fructose and glucose contents were positively correlated with total F+G content, whereas HMF and diastase activity were negatively correlated. Furthermore, positive correlations were identified between electrical conductivity and proline and diastase levels in the honey samples. The results of Pearson's correlation analysis are presented in Table-4.

**Table 1. Results of melissopalynological analysis of honey samples**

Sample Number Family (%)	Z1	Z2	Z3	Z4	Z5	Z6	Z7	Z8	Z9
Fagaceae ( <i>Castanea sativa</i> )	76.5	86.5	72.5	85.5	85	85.5	86	76	87
Fabaceae	5	2.5	10	2.5	5	5.5	3	3	1
Cistaceae	4.5	2		2.5	3		3	1	4
Rosaceae	3		1	2	3			2	
Ericaceae ( <i>Rhododendron sp.</i> )	2		3		1	2			
Brassicaceae	2			2.5		1		10	1
Malvaceae	2.5	1.5	4	3	2	2	3	2	4
Cornaceae	1.5						2		2
Adoxaceae		2.5	5.5					5	
Asteraceae		1		1		2			
Ranunculaceae		2	2						
Tamaricaceae			1						
Apiaceae						1	1		
Others	3	2	1	1	1	1	2	1	1

**Table 2. Results of the physicochemical analyses of honey samples**

Sample Number	Moisture (%)	Electrical Conductivity (mS/cm)	Fructose (%)	Glucose (%)	F+G (%)	F/G Ratio	Sucrose (%)	Maltose (%)	HMF (mg/kg)	Diastase (DN)	Proline (mg/kg)
Z1	15.20±0.40	1.31±0.08	34.40±1.48	29.09±1.43	63.49	1.18	ND	3.17±0.21	0.25±0.04	38.9±2.4	878.3±60.2
Z2	16.50±0.00	1.63±0.10	38.11±1.64	26.77±1.31	64.88	1.42	ND	2.76±0.18	0.56±0.09	36.4±2.3	1084.6±74.4
Z3	17.06±0.44	1.16±0.07	34.92±1.50	30.35±1.49	65.27	1.15	ND	2.5±0.16	3.7±0.59	18.2±1.1	803.5±55.1
Z4	18.84±0.49	1.06±0.06	38.75±1.67	30.85±1.51	69.60	1.26	ND	2.5±0.16	ND	23.6±1.5	647±44.4
Z5	15.36±0.40	1.18±0.07	43.53±1.75	35.64±1.87	79.17	1.22	ND	3.35±0.22	7.2±1.15	21.3±1.3	810.5±55.6
Z6	17.50±0.45	1.43±0.09	39.73±1.71	28.65±1.40	68.38	1.39	ND	2.60±0.17	ND	38±2.4	1092.3±74.9
Z7	15.00±0.39	1.52±0.09	36.12±1.55	30.73±1.51	66.85	1.18	ND	2.64±0.17	1.32±0.21	36.9±2.3	1172±80
Z8	19.03±0.49	1.53±0.09	33.16±1.43	28.79±1.41	61.95	1.15	0.16±0.01	3.83±0.25	1.1±0.18	30.6±1.9	831±57
Z9	16.93±0.44	1.12±0.07	34.86±1.50	26.99±1.32	61.85	1.29	ND	1.44±0.09	ND	19.4±1.2	650±45
Limits	≤ 20%	≥ 0.8			≥ 60%	1-1.85	≤ 5%	≤ 4%	≤ 40	≥ 8	≥ 500

ND: Not detected.

**Table 3. Descriptive statistics of the analysis parameters for honey samples**

Physicochemical properties	Mean ± SD	Median (Min - Max)	Shapiro-Wilk (p)
Moisture (%)	16.82 ± 1.42	16.93 (15.00 – 19.03)	0.386
Electrical Conductivity (mS/cm)	1.32 ± 0.20	1.31 (1.06 – 1.63)	0.403
Fructose (%)	36.71 ± 3.21	36.12 (33.16 – 43.53)	0.458
Glucose (%)	29.32 ± 2.64	29.09 (26.77 – 35.64)	0.153
F+G (%)	66.03 ± 5.21	64.88 (61.85 – 79.17)	0.055
F/G Ratio	1.26 ± 0.10	1.26 (1.15 – 1.42)	0.142
Maltose (%)	2.80 ± 0.66	2.64 (1.44 – 3.83)	0.545
HMF (mg/kg)	2.36 ± 2.67	1.21 (0.25 – 7.20)	0.073
Diastase (DN)	29.48 ± 8.63	30.60 (18.20 – 38.90)	0.079
Proline (mg/kg)	877.74 ± 189.58	854.65 (647.00 – 1172.00)	0.291

Normality was verified via Shapiro–Wilk test ( $p > 0.05$ ). Data are presented as mean ± standard deviation.  $n = 9$

**Table 4. Pearson correlation matrix of physicochemical parameters of honey samples**

Parameters	1	2	3	4	5	6	7	8	9	10
1 Moisture										
2 Electrical Conductivity	-0.117									
3 Fructose	-0.203	-0.170								
4 Glucose	-0.291	-0.386	0.624							
5 F+G	-0.269	-0.296	0.922**	0.878**						
6 F/G Ratio	0.088	0.260	0.435	-0.433	0.051					
7 Maltose	0.027	0.423	0.072	0.383	0.234	-0.366				
8 HMF	-0.178	-0.717	0.763	0.908*	0.896*	-0.175	0.064			
9 Diastase	-0.258	0.783*	-0.102	-0.347	-0.235	0.286	0.341	-0.824*		
10 Proline	-0.427	0.829**	0.106	-0.145	-0.007	0.308	0.216	-0.495	0.792*	

Pearson correlation coefficients are shown. \* $p < 0.05$ , \*\* $p < 0.01$  (two-tailed).  $n = 9$

## DISCUSSION

According to the TFC-HC, honey labeled as chestnut honey must contain at least 70% *Castanea sativa* pollen in its total pollen profile. In this study, the *Castanea sativa* pollen ratio in all analyzed honey samples exceeded the limits specified in the communiqué. A study by Özkök and Bayram (2021) on the melissopalynological characteristics of chestnut honeys from the Zonguldak region identified pollen from various plant taxa. That study identified *Castanea sativa* (Fagaceae) as the dominant pollen, consistent with our findings. Pollen types belonging to the families Fagaceae, Fabaceae, Cistaceae, Rosaceae, Ericaceae, Asteraceae, and Apiaceae, which were identified as secondary pollen sources in their research, were also present in the samples examined in our study.

Conversely, certain differences in pollen families were observed between the findings of Özkök and Bayram (2021) and the present study. These discrepancies may stem from the floristic structure of the apiary locations and the diversity of the surrounding vegetation. The TFC-HC establishes specific limits for parameters such as moisture, electrical conductivity, fructose, glucose, F+G, F/G ratio, maltose, HMF, diastase activity, and proline. Literature reviews of chestnut honey produced in Türkiye show that Küçük et al. (2007) reported average values for samples from the Black Sea region as  $19.7 \pm 1.7\%$  for moisture,  $66.8 \pm 6.2\%$  for F+G,  $28.6 \pm 1.9$  mg/kg for HMF, and  $17.7 \pm 1.4$  DN for diastase activity.

Kabakçı et al. (2012) reported values for samples from Ordu and Giresun as 17.46% and 16.02% for moisture, 0.84 and 1.20 mS/cm for electrical conductivity, 61.78% and 59.23% for F+G, 4.96 and 7.61 mg/kg for HMF, and 8.32 and 9.33 DN for diastase activity, respectively. Dağ (2017) reported that chestnut honey samples produced in the Black Sea Region exhibited a moisture of  $18.13 \pm 1.53\%$ , a total F+G of  $71.15 \pm 2.26\%$ , and F/G ratio of  $1.44 \pm 0.05$ . Furthermore, the researchers determined the HMF concentration to be  $0.57 \pm 0.09$  mg/kg and the diastase activity to be  $14.20 \pm 2.87$  DN. Silici (2018) determined the average parameters for chestnut honeys from different regions of Türkiye as follows: moisture  $17.31 \pm 0.83\%$ , electrical conductivity  $0.89 \pm 0.36$  mS/cm, fructose  $37.09 \pm 2.29\%$ , glucose  $30.41 \pm 4.08\%$ , F+G  $67.50 \pm 6.12\%$ , F/G ratio  $1.23 \pm 0.12$ , maltose  $1.99 \pm 0.28\%$ , HMF  $22.38 \pm 17.55$  mg/kg, diastase activity  $15.65 \pm 3.60$  DN, and proline  $740 \pm 94.44$  mg/kg.

Avşar et al. (2023) reported that in their study on chestnut honeys from the Sinop region, the moisture ranged from 18.04 to 19.40%, electrical conductivity from 0.25 to 0.28 mS/cm, fructose content from

39.90 to 40.60%, glucose from 22.00 to 25.30%, F+G from 62.10 to 65.80%, F/G ratio from 1.59 to 1.76, sucrose content from 0.05 to 0.39%, maltose content from 0.05 to 0.08%, HMF content from 1.22 to 3.44 mg/kg, diastase activity from 13.42 to 20.37 DN, and proline from 702.94 to 855.49 mg/kg. Saral (2023) identified parameters for Artvin chestnut honeys as moisture  $18.41 \pm 0.50\%$ , electrical conductivity  $0.82 \pm 0.19$  mS/cm, fructose  $40.30 \pm 1.37\%$ , glucose  $30.41 \pm 1.66\%$ , F+G  $70.71 \pm 3.03\%$ , F/G ratio  $1.32 \pm 0.82$ , diastase activity  $22.79 \pm 2.67$  DN, and proline  $1062.90 \pm 112.22$  mg/kg.

Yoldaş et al. (2023) reported values for Düzce chestnut honeys as moisture  $18.40 \pm 2.86\%$ , electrical conductivity  $1 \pm 0.48$  mS/cm, F+G  $58.75 \pm 9.80\%$ , F/G ratio  $1.40 \pm 0.17$ , maltose  $1.75 \pm 0.83\%$ , HMF  $1.63 \pm 0.40$  mg/kg, diastase activity  $15.28 \pm 11.11$  DN, and proline  $736.80 \pm 286.24$  mg/kg. Uçurum et al. (2024) obtained results for Zonguldak and Bursa honeys with moisture  $17.40 \pm 1.20\%$ , electrical conductivity  $1.43 \pm 0.33$  mS/cm, fructose  $35.83 \pm 1.87\%$ , glucose  $26.66 \pm 2.62\%$ , F+G  $66.12 \pm 3.45\%$ , F/G ratio  $1.40 \pm 0.15$ , maltose  $2.73 \pm 1.12\%$ , and proline  $1015.60 \pm 362.89$  mg/kg. The values obtained in our study for moisture  $16.82 \pm 1.42\%$ , electrical conductivity  $1.32 \pm 0.20$  mS/cm, fructose  $36.71 \pm 3.21\%$ , glucose  $29.32 \pm 2.64\%$ , F+G  $66.03 \pm 5.21\%$ , and the F/G ratio  $1.26 \pm 0.10$  are in alignment with previous studies on chestnut honeys from various regions of Türkiye.

The high phenolic content of chestnut honeys is closely correlated with their dark color and enhanced biological activity. These compounds contribute significantly to antioxidant capacity. Electrical conductivity is primarily related to the mineral and organic acid content of honey; however, it may also reflect, to some extent, the presence of other bioactive compounds. The relatively high electrical conductivity values obtained in this study are consistent with the literature and may indicate a rich mineral composition, which is characteristic of chestnut honey (Kolaylı et al. 2020, Kanbur et al. 2021).

In our study, the positive correlation observed between fructose, glucose, and F+G confirms that the total reducing sugar amount primarily originates from these two monosaccharides. The negative correlation between HMF and diastase activity reflects qualitative changes in honey resulting from thermal processing and storage conditions. Furthermore, the positive correlation among electrical conductivity, proline, and diastase activity is a significant indicator of the botanical origin and overall quality of honey samples. In summary, the correlation analyses yielded results consistent with existing literature. Notably, the low moisture suggests that the honey is well matured with a low

risk of fermentation. The observed low HMF values ( $2.36 \pm 2.67$  mg/kg) and high diastase activity ( $29.48 \pm 8.63$  DN) demonstrate that the samples were not subjected to thermal processing. This is particularly characteristic of chestnut honey, which, due to its high fructose-to-glucose ratio ( $1.26 \pm 0.10$ ), has a low tendency to crystallize and therefore typically does not require heating to liquefy. Consequently, chestnut honey is less likely to undergo thermal degradation, which helps maintain its natural biochemical integrity, preserve enzyme activity, and minimize HMF formation (Celebi et al. 2026).

The high proline content of  $877.74 \pm 189.58$  mg/kg further confirms that the honey is a natural, mature product. Our results also show high similarity with studies examining the physicochemical properties of chestnut honeys produced in Slovenia, Croatia, Greece, and Italy (Kropf et al. 2010, Šarić et al. 2008, Thrasyvoulou and Manikis 1995, Fallico et al. 2004). In conclusion, a comprehensive evaluation of all analyzed parameters indicates that the samples in our study remain within the limits established by the TFC-HC. Furthermore, the findings indicate that these samples exhibit characteristics similar to those of high-quality chestnut honeys reported in the scientific literature.

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