

## Assessment of Palynological Characterization and Total Phenol-Flavonoid Content of Some Honeys from Ordu in Turkey

İlginç KIZILPINAR TEMIZER<sup>1\*</sup>, Aytaç GÜDER<sup>1</sup>, Zafer TÜRKMEN<sup>2</sup>

<sup>1</sup> Giresun University, Vocational High School of Health Services, Giresun, 28200, Turkey

<sup>2</sup> Giresun University, Science and Art Faculty, Giresun, 28200, Turkey

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### Abstract

Honey is one of the important honey bee products and varies total phenolic and flavonoid contents depending on mainly its floral source. In this study, the total phenolic-flavonoid content and pollen composition of fifteen honey samples were investigated from Ordu in Turkey. According to melissopalynological (qualitative) analysis, eleven honey samples were unifloral (*Castanea sativa* (9); Rosaceae (1) and *Onobrychis* spp.(1)), while four samples were multifloral. The forty-two pollen types from the samples were identified. The melissopalynological (quantitative) analysis based on Maurizio's Class demonstrated that one in Class II, three in Class III, one in Class IV and ten in Class V. Total phenolic and total flavonoid amounts in these honeys varied from 32.5-171.05 mg GAE (gallic acid equivalent)/100g, 1.65-38.75 mg CAE (catechin equivalent)/100g, respectively. According to this study the highest flavonoid levels obtained from Sample 9 (multifloral) and the highest phenolic contents were shown in sample 1 (*C. sativa*). This study contributes to literature about Ordu honeys.

**Keywords:** flavonoid, honey, phenolic, Ordu

### Türkiye'nin Ordu iline ait Bazı Balların Palinolojik Karakterizasyonu ve Toplam Fenol-Flavonoid İçeriğinin Değerlendirilmesi

#### Öz

Bal, en önemli arı ürünlerinden biridir ve total fenol ve flavonoid miktarı floral kaynağına göre değişkenlik gösterir. Bu çalışmada Türkiye'nin Ordu ilinden alınan 15 bal örneğinin toplam fenol, toplam flavonoid ve polen dağılımı incelenmiştir. Melissopalinojik (kalitatif) analizlere göre 11 bal unifloral (*Castanea sativa* (9); Rosaceae (1) and *Onobrychis* spp.(1)), dört örnek multifloraldır. Örneklerden 42 polen tipi tanımlanmıştır. Maurizio's Sınıflandırmasına dayanarak yapılan mellissopalinojik (kalitatif) analizler 1 tane Class II, 3 tane Class III, 1 tane Class IV ve 10 tane Class V olduğunu göstermiştir. Bu ballardaki toplam fenol ve flavonoid içerikleri sırasıyla 32.5-171.05 mg GAE/100g, 1.65-38.75 mg CAE/100g aralığında değişmektedir. Bu çalışmaya göre en yüksek flavonoid seviyesi örnek 9 (multifloral)'dan elde edilmiştir ve en yüksek fenol içeriği örnek 1 (*C. sativa*)'de gözlenmiştir. Bu çalışma literature Ordu balları hakkında katkı sağlamaktadır.

**Anahtar Kelimeler:** Flavonoid, bal, fenol, Ordu

## 1. Introduction

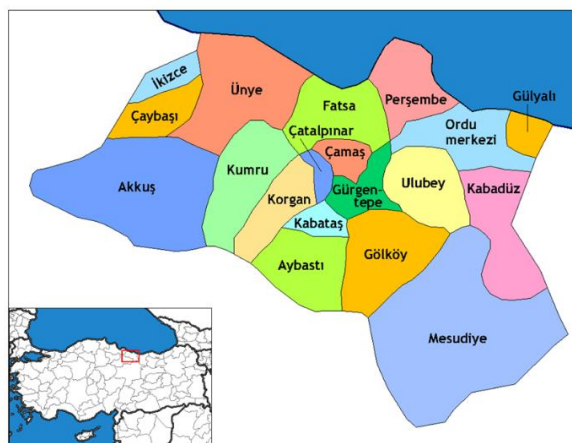
Honey is defined as a naturally sweet mixture which consists of glucose, fructose and other substances (proteins, polyphenolic compounds, organic acids, amino acids, minerals etc.) (White and Doner, 1980). Polyphenolics, mainly phenolic acids and

flavonoids, were defined as the mainly components account for the health-promoting features of honey (Kečkeš et al., 2013). Many authors have shown that the varied nutritional quality, medicinal and sensory properties of honeys are due to botanic origin, honey is never completely the same another one (Rababah et al., 2014; Al-Farsi et al., 2018;

Kavanagh et al., 2019).

The optimum climate and vegetation conditions of Ordu are allowed a long duration to make beekeeping activities. Ordu is situated at 40°18'-41°08'N, 36°52'-38°12' E. It is located in the A6 square (Figure 1) and is characterized by euxine and colchic sub-provinces of Euro–Siberian floristic region. It

consists of mainly deciduous broadleaf trees and evergreen shrubs (Davis 1965-1985). Indeed, it has an enormously wild forest area with rich bee flora. Asteraceae, Brassicaceae, Fabaceae families and *C. sativa* are very important nectar or pollen resource for *Apis mellifera* (honey bee) in Ordu (Deveci et al., 2012).



**Fig. 1.** Geographical location of Ordu city

([https://commons.wikimedia.org/wiki/File:Ordu\\_districts.png](https://commons.wikimedia.org/wiki/File:Ordu_districts.png))

Mellissopalynological analysis is a useful instrument for demonstrating the sources of nectar and pollen. Therefore, in the expanding global market, pollen analysis of honey has become a more important task than ever. Despite the importance of beekeeping in Ordu, its honeys are insufficiently-studied. This study aims at evaluating the some authenticity criteria of 15 honey samples from Ordu. For this purpose, pollen analysis, total phenolic content and total flavonoid content were performed. In addition, honey classified Maurizio's classification.

## 2. Experimental

### 2.1. Materials and Methods

Honey samples were collected between August-September of 2015 from different

localities of Ordu (Turkey) which is part of the north-east region (Table 1).

Firstly, we give the fundamental definition and properties for this sequence.

**Table 1.** Sample no and locations of honey bee pollens collected from Ordu

1	Sayacabaşı (Ulubey)	6	Perşembe	11	Ulubey
2	Boztepe	7	Merkez	12	Yokuşdibi
3	Kabadüz	8	Ünye	13	Günören (Merkez)
4	Gölköy	9	Merkez	14	Günören (Merkez)
5	Gülyalı	10	Kabadüz	15	Merkez

## 2.2. Melissopalynological Analysis

Preparation of honey sample for qualitative and quantitative melissopalynological analysis was performed in accordance with the international method (Maurizio and Hodges, 1951; Lieux 1972; Louveaux and Maurizio, 1978). The total pollen number (TPN) of all samples was calculated according to the method described by Moar (1985). Classification of honey samples by TPN-10 values was done according to Maurizio (1975) Group I (<20,000 pollen grains); Group II (20,000–100,000); Group III (100,000–500,000); Group IV (500,000–1,000,000); and Group V (>1,000,000).

The terms used for the evolution of the frequency classes in honeys were: dominant pollen (>45%), secondary pollen (16-45%), minor pollen (3-15%), and trace pollen (<3%) (Louveaux and Maurizio, 1978).

## 2.3. Antioxidant Analyses

### 2.3.1. Determination of total flavonoid content (TFC)

The total flavonoid content of the samples were determined according to the colorimetric method described by Chung et al. (2002) with slight modifications. Absolute ethanol (1.5 mL) mixed samples (0.5 mL) were added to 0.1 mL of 10%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$  solution and 0.1 mL of 1.0 mol  $\text{L}^{-1}$  potassium acetate. The final volume was increased to 5 mL with deionized water. The mixture was left at room temperature for 30 min and the absorbance was calculated at 415 nm by a spectrophotometer (Optizen Pop UV / Vis Single Beam, Korea). Total flavonoid content was expressed as micrograms of CAE that were obtained from a standard graph ( $R^2=0.9979$ ).

### 2.3.2. Determination of total phenolic contents (TPC)

The total phenolic content of the samples was analyzed by the Folin and Ciocalteu's phenol reagent (Folin C) colorimetric method described by Slinkard and Singleton (1977). Sample solutions (0.5 mL) were mixed with 7.0 mL of distilled water and subsequently with Folin C reagent (0.5 mL). After 3 min a  $\text{Na}_2\text{CO}_3$  solution (3.0 mL, 2.0 %) was added into the mixture. The color developed for 1 hour and the absorbance was measured at 760 nm by a spectrophotometer (Optizen Pop UV / Vis Single Beam). Gallic acid was used as the standard, and the total phenolic content was expressed as micrograms of GAE by using an equation that was obtained from a standard gallic acid graph ( $R^2=0.9995$ ).

## 2.4. Statistical Analysis

The relationship between total phenolic and flavonoid content of samples was determined by Spearman correlation tests. Analyses were done with SPSS (version 15.0, SPSS Inc., Chicago, IL, USA) for Windows.

## 3. Findings

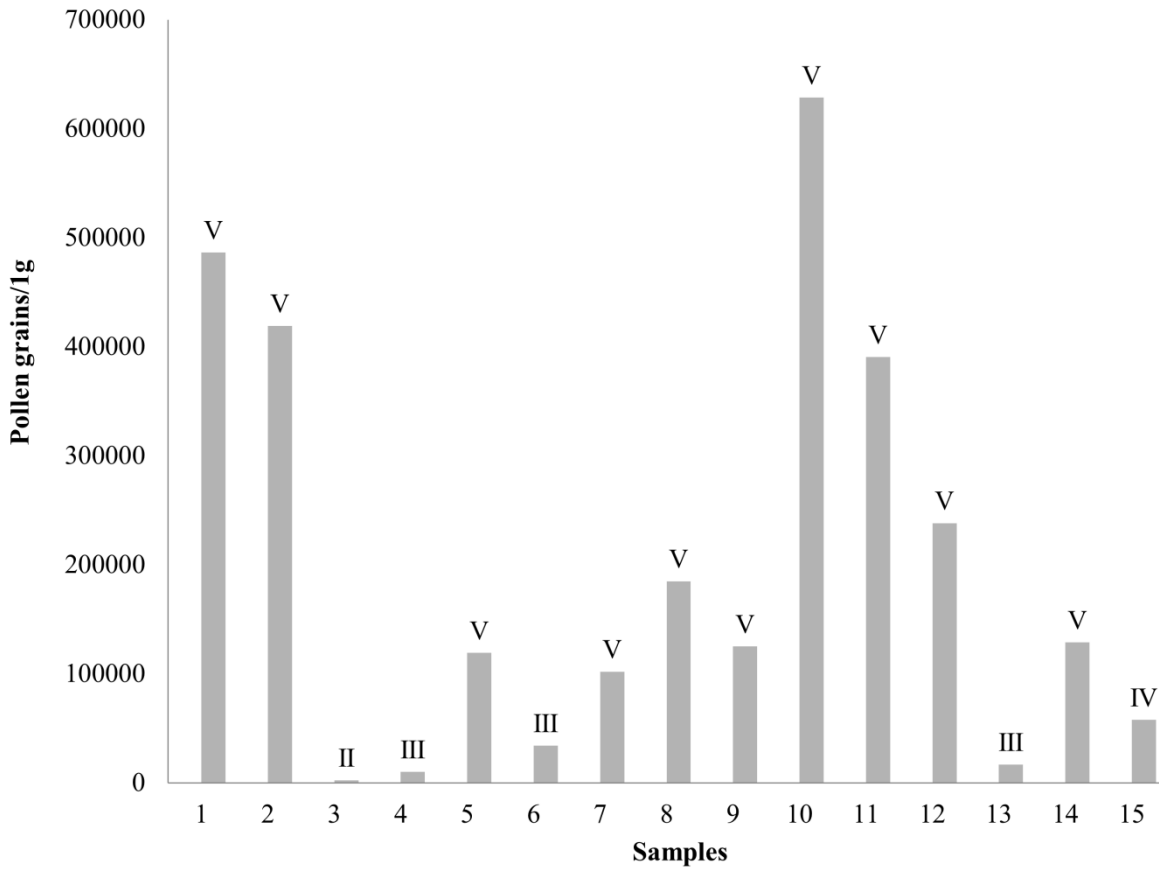
In this study, the pollen spectra of the randomly selected fifteen honey samples collected from different locations in Ordu (Turkey) were analyzed, including the identification of total phenolic and flavonoid contents. The pollen frequency classes are given in Table 2.

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**Table 2. Pollen types and their frequency class distributions in the 15 samples**

Samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>Acer</i>				T											
<i>Anchusa</i>											S				
<b>Apiaceae</b>		T	T	T	T			T	T		T	T			T
<b>Asteraceae-echinate typ</b>		T	T	M		T		S	M		T	T	T	M	
<b>Asteraceae-lakun typ</b>				T		T	M	T		T		T		T	
<b>Asteraceae-scabrat typ</b>									T		T				
<b>Brassicaceae</b>								M			T	T		M	
<b>Boraginaceae</b>		T		M			M		T		T	T	T	M	T
<i>Campanula</i>		T		T											T
<b>Caryophyllaceae</b>								T		T	T			M	
<i>Castanea sativa</i>	D	D			D	D	D	M		D	D	D	D		
<i>Centraurea</i>								T	T		T	T		M	
<b>Chenopodiaceae</b>		T													
<b>Cistaceae</b>				M	T			M			T			T	T
<i>Daucus</i>	T											T			
<b>Dipsacaceae</b>		T						T	M						
<i>Echium</i>								T				T			
<b>Fabaceae</b>	T	T	T	M	T		M	M	S	T	T	M	S	M	S
<i>Juglans</i>		T							M		T	T			T
<b>Lamiaceae</b>				M							T			T	T
<i>Lonicera</i>									M					S	
<i>Medicago</i>			T	M		T		M						T	T
<b>Oleaceae</b>		T							T		T				
<i>Onobrychis</i>			D	S	T		S	S		T	M			M	T
<i>Plantago</i>															T
<i>Pseudoacacia</i>									M				M		
<b>Poaceae</b>	T	T		T	T	T		T		T		T			T
<i>Rhododendron</i>											T		T		M
<b>Rosaceae</b>		T	T	M		T		T		T	T	T		M	D
<i>Rumex</i>								T			T	T		T	T
<i>Salix</i>	T			M										T	M
<i>Taraxacum</i>											T				
<i>Tilia</i>									S	T	T				
<i>Trifolium</i>			T	T		T	M	M						M	T
<b>Undetermined</b>				T	T					T	T	T		T	T

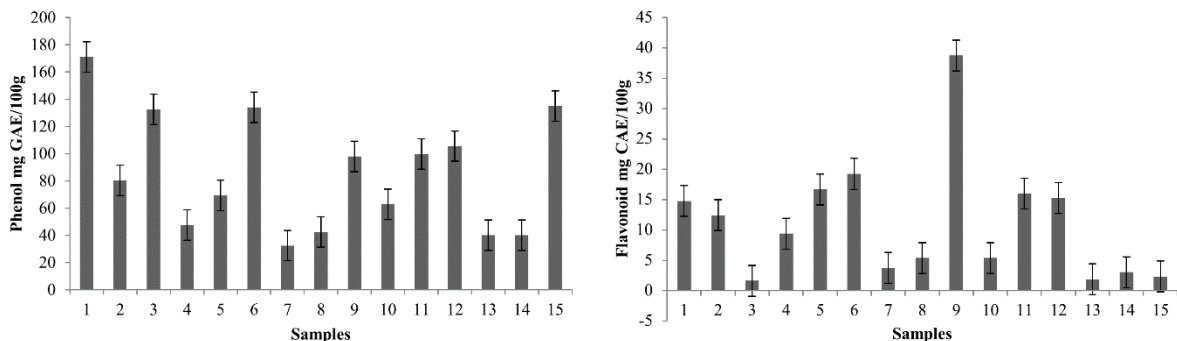
(D: >45%, S:16-45%, M: 3-15% and T:<3%)



**Fig. 2.** Pollen richness according to Maurizio's Class (The Roman numbers indicate the Class).

The total phenolic and flavonoid contents range from 32.5-171.05 mg GAE/100g and 1.65-38.75 mg CAE/100g, respectively. Sample 1 has the highest total phenolic content and sample 7 has the lowest contents. In addition, sample 9

demonstrates the highest flavonoid content and sample 3 has the lowest content (Figure 2). There was no significant correlation between the total phenolic and flavonoid content ( $p>0.05$ ).



**Fig. 3.** Total phenolic and flavonoid contents of samples

#### 4. Discussion

In this study it was found that all of the samples were natural honey in terms of pollen characteristics. The result of pollen analysis indicated that the honey samples were plentiful in different taxa but in low percentage. In addition, Mellisopalynological analysis of honey samples showed a slightly variability between samples from different localities of Ordu. The botanical classification of honeys indicates that 9 would be classified as Chestnut (*C. sativa*) honey, one as *Onobrychis* honey, one as Rosaceae honey and the rest as multifloral honey. Results obtained the study determined that *C. sativa* is mainly nectar and pollen sources in Ordu beekeeping activities. The *C. sativa* and *Rhododendron* spp. pollen types may be regarded as geographical markers for the Ordu and Euro-Siberian region of Turkey, though the *Rhododendron* pollen type was found only in three samples. However, beekeepers may not choose *Rhododendron* because it contains andromedotoxin that can cause serious side effects in humans (Michie et al., 2011). Previous researchers have investigated Chestnut honey have noted that *C. sativa* pollen is strongly over represented in honey (Louveaux, et al., 1978; Ramos et al., 2002). The results obtained from this study are in agreement with these researchers (78% Class V, Maurizio's). *Onobrychis* spp. honey, from unifloral honeys based on pollen grains, is represented in Class II, while Rosaceae honey is found in Class IV. *Onobrychis* spp. in inner and east Anatolia from Turkey is the important plant for honey bee (Özbek, 2011) and indicates traveler beekeeping in Ordu. Pollen concentrations of multifloral honeys are assigned to Class V (75%) and Class III (25%).

The value for total phenolic content in studied samples were usually higher than the results declared by other searches (Lachman 2010; Özbek, 2011; Šarić et al., 2012; Pontis et al., 2014; Kavanagh et al. 2019; Selvaraju et al. 2019). However the phenolic content of these samples was lower than samples from Black Sea region of Turkey (Kolaylı et al., 2008). Total flavonoid amount of samples are similar compared to the results reported in the literature (Meda et al., 2005; Šarić et al., 2012) and lower than Moniruzzaman et al. (2014) and Al-Farsi et al. (2018). The total phenolic amount of our findings was similar to Boussaid et al. (2018), while the total flavonoid content was higher than Boussaid et al. (2018) and Selvaraju et al. (2019).

Analyses of the 15 honey samples from Ordu city of Turkey revealed the occurrence of different types of pollens from 42 plant taxa. In addition, eleven honey samples were unifloral and the rest were multifloral. Honey samples have different phenolic-flavonoid content as evidenced by the varied pollen spectra. The results obtained from study had significant total phenolic and flavonoid contents. Therefore, it may be regarded as a perfect source of phenolic flavonoid source. There are no reports available concerning the pollen spectra, total phenolic content and total flavonoid content of Ordu honeys that make the present work being original in this field. In the next study, the honey samples obtained from Ordu, the phenolic and flavonoid component(s) can be detected by using different chromatographic techniques so we can evaluate the isolated dominant active component(s) in honey samples clearly. Then, biochemical and pharmacological activities of these component(s) may be identified via in vivo experiments.

## 5. Acknowledgements

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