

Some Characteristic Properties of Chestnut and Rhododendron Honeys in Turkey

Türkiye'deki Kestane ve Ormangülü Ballarının Bazı Karakteristik Özellikleri

Research Article

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ABSTRACT

Chestnut (*Castanea sativa* Mill.) and Rhododendron (*Rhododendron* spp. L.) (mad honey) honeys are produced generally in Black Sea Region in Turkey and both of them are the special honeys because of their organic component content and known their high antioxidant capacity. In the first step of this study we researched the melissopalynological differentiation of the chestnut and rhododendron honeys and then in the second step we determined the chemical compounds and sugar content of the rhododendron, chestnut and mixed chestnut&rhododendron honeys. For this purpose total 18 honey samples were collected from 4 different districts from Black Sea Region of Turkey and melissopalynological analyses were done by microscope. Chemical composition and sugar content (fructose&glucose) were determined by High Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS). After melissopalynological analyses were obtained 10 monofloral chestnut, 2 monofloral rhododendron and 6 mixed chestnut&rhododendron honeys. As a result of sugar analysis with HPLC, F/G rates were found between 1.17 and 1.80. GC-MS chemical substance analyses of honeys revealed alcohols, aldehydes, ketones, aliphatic acids and their esters, carboxylic acids and their esters and flavanonoids.

Key Words

Chestnut honey, rhododendron honey, melissopalynological analysis, sugar analysis, chemical compounds.

ÖZ

Türkiye'de genellikle Karadeniz bölgesinde üretilen kestane (*Castanea sativa* Mill.) ve ormangülü (*Rhododendron* spp. L.) (delibal) balları içerdiği organik bileşenler ve yüksek antioksidan seviyeleri ile bilinen önemli ballardır. Bu çalışmanın ilk aşamasında kestane ve ormangülü ballarının melissopalinojik farklılıkları araştırılmış, ikinci aşamasında ise ormangülü, kestane ve kestane ormangülü karışık balların şeker içerikleri ve kimyasal bileşenleri belirlenmiştir. Bu amaçla Türkiye'nin Karadeniz Bölgesinde, 4 farklı yerden toplamda 18 bal örneği toplanmıştır ve mikroskop ile melissopalinojik analizleri yapılmıştır. Kimyasal bileşim ve şeker içeriği (fruktoz&glikoz) HPLC ve GC-MS ile belirlenmiştir. Melissopalinojik analizler sonrasında 10 balın monofloral kestane balı, 2'sinin monofloral ormangülü ve 6'sının da kestane ormangülü karışık ballar olduğu saptanmıştır. HPLC ile yapılan şeker analizleri sonucunda F/G oranının 1.17 ve 1.80 arasında olduğu, GC-MS kimyasal analizleri sonucunda da balların alkoller, aldehydler, ketonlar, alifatik asit ve esterleri, carboksilik asit ve esterleri ile flavanoidleri içerdiği ortaya çıkarılmıştır.

Anahtar Kelimeler

Kestane balı, deli bal, melissopalinojik analizler, şeker analizleri, kimyasal bileşikler.

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INTRODUCTION

Chestnut (*Castanea sativa* Mill.) and Rhododendron (*Rhododendron* spp. L.) (mad honey) honeys are found generally in Black Sea Region in Turkey and both of them are the special honeys for their organic component content and known their high antioxidant activity.

Chestnut plant is one of the best sources of nectar and pollen for honeybees. Chestnut honey with dark color and bitter taste, can stay in a liquid state for a long time because of its slow crystallization rate [1,2].

Mad honey, produced by honeybees from the nectars of *Rhododendron* genus (*R. ponticum* and *R. luteum*) flowers. *R. ponticum* and *R. luteum* plants, which are belongs to Ericaceae family, grow mainly in the Black Sea Region of Turkey, Japan, Nepal, Brazil, Europe and some parts of North America [3]. This honey's taste is bitter because of its slightly sharp taste and most of them contains toxins which are called grayanotoxins and they can be toxic when their consumption. So people use generally "Mad Honey" name for this honey due to subsequent consumption effects. On the other hand this honey is widely used in indigenous medicine [2].

In *Rhododendron* genus, 208 compounds have been isolated, composed of mostly flavonoids and diterpenoids [4]. Most of those diterpenoids are grayane-type diterpenoids, polyhydroxylated cyclic hydrocarbons that do not contain nitrogen [5]. It

is reported that mad honey intoxication is largely associated with lipid-soluble grayanotoxins (GTxs) similar to the alkaloids veratridine, acotinine and batrachotoxin [6]. GTxs tend to bind to the activated state of sodium channels and cause persistent activation at resting membrane potential. They lead to blockage of sodium channel inactivation and shift of the voltage dependence of activation to more negative potentials [2,7].

In this study we researched the melissopaynological differentiation of the chestnut and rhododendron honeys and then we determined the chemical compounds and sugar content of the rhododendron, chestnut and mixed chestnut rhododendron honeys

MATERIALS and METHODS

Collection of Honey Samples

Honey samples were provided by beekeepers. Total 18 honey samples were collected from 4 different districts [Bartın (n=10); Kastamonu (n=1); İstanbul (n=1); Düzce (n=6)] from Black Sea Region of Turkey (Figure 1). Pollen analysis was performed to authenticate the botanical origin of chestnut and rhododendron honeys.

Microscopic Analysis of Honeys

For microscopic analysis Wodehouse (1935) [8] and Sorkun (2008) [9] methods were accepted and honey preparations were examined by Olympus CX41 microscope.

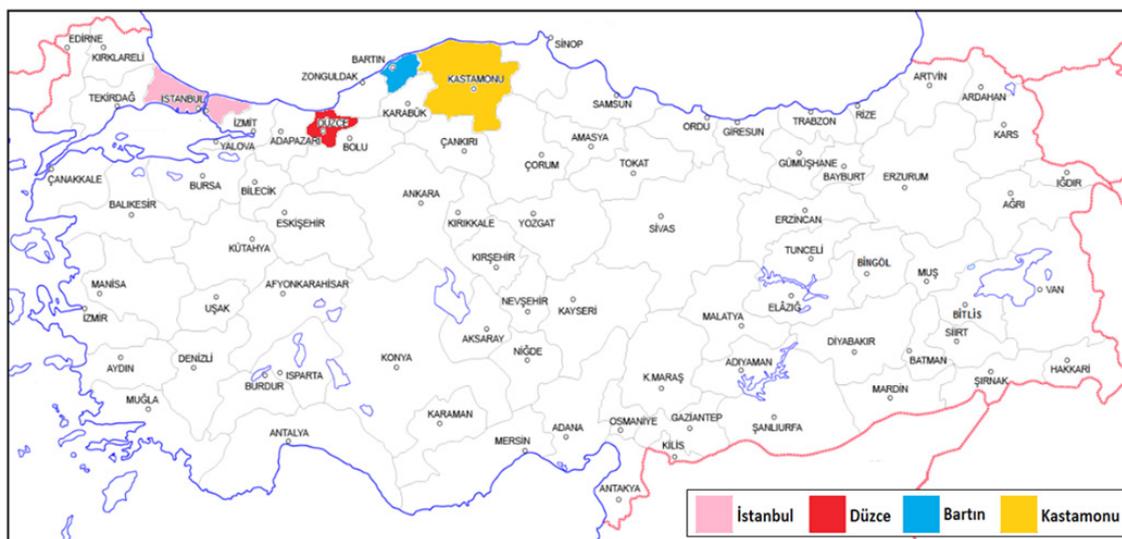


Figure 1. The regions where honey samples were collected.

Preparates from Honey Samples

Preparates to identify in 10 grams of honey are obtained as follows:

500 grams of stock honey was well stirred with a sterile glass stick and 10 grams of it was separated for obtain preparats. Then the sample and 20 ml distilled water mixed in a tube and left in a water bath of 45°C for 30-45 minutes. Then this melted honey mixture was centrifuged in 3500 rpm for 45 minutes. Water in centrifuged tubes was removed and tubes were left upside down on a drying mat for full drainage. The material was taken from the bottom of the tube and plated on a lam with basic fucsin-glycerin gelatin mixture.

Basic fucsin-glycerin-gelatine mixture and honey were taken with the edge of a sterile needle was transferred to a microscope slide and put on a hotplate set at 40°C. When the gelatine was melted, 18x18 mm cover slips were placed on the samples. Pollen slides were researched with Olympus CX41 microscope and immersion objective (x100) was used for identification of pollens. During microscopic studies all the area, which is 18x18 mm, was checked. 200 pollen was counted for every sample and determined pollen types according to their botanical origin.

Relevant sources were used in the identification of the pollen were from Persano Oddo and Piro (2004) [10], Özkök Tüylü and Sorkun (2007) [11] and Sorkun (2008) [9] as well as reference preparats

Determination of the Botanical Originals of Honey Samples

The determination of the botanical origin is based on the relative frequencies of nectariferous species' pollen types. The frequency classes of pollen grains were given as predominant (>45%), secondary pollen (15-45%), important minor pollen (3-15%) and minor pollen (<3%) [12].

Generally a honey can be defined as unifloral if the "characteristic" pollen (e.g. *Brassica* in rape honey) exceeds 45%. These are general guidelines but many pollen types are underrepresented (*Robinia pseudoacacia*, *Citrus* spp., *Tilia* spp.) or over-represented (*Castanea sativa*, *Eucaliptus* spp.). For instance, to characterize acacia honey as unifloral, *R. pseudoacacia* pollen must be over 15%, citrus

must have at least 10% of *Citrus* spp. pollen while, for chestnut honey, a content of 70-90% of *Castanea* pollen is required to classify honey as unifloral [12-14].

High Performance Liquid Chromatography (HPLC) Sugar Analysis

Bogdanov and Baumann (1988) [15] method and HPLC (Agilent 1200 Series) were used for the determination of fructose and glucose at honey samples. According to this method 5 g honey was weighed into a beaker and dissolved in 40 ml water. Pipetted 25 ml of methanol into a 100 ml volumetric flask and transferred the honey solution quantitatively to the flask. Filled to the mark with water. Poured through a membrane filter and collected in sample vials. Also fructose (2 g) and glucose (1.5 g) standards were prepared same way. 80 volumes of acetonitrile to 20 volumes of water mix was used as mobil phase. Flow rate was 1.3 ml/min at constant temperature of 30°C.

Gas Chromatography-Mass Spectrometry (GC-MS) Chemical Compounds Analysis

GC-MS was used for chemical compound analysis of the honey samples. 5 g honey dissolved in 5 ml methanol and mixed for 1 minute by vortex. Next centrifuged at 3500 rpm for 15 minutes. Then upper phase was filtered to vials and 1 µl solution injected to GC-MS. A GC 6890N from Hewlett-Packard (Palo Alto, CA, USA) coupled with mass detector (MS 5973 Hewlett-Packard) was used for the analysis of honey samples. Experimental conditions of GC-MS system were as follows: DB 5MS column (30 mx0.25 mm and 0.25 µm of film thickness) was used and flow rate of mobile phase (He) was set at 0.7 ml/min. In the gas chromatography part, temperature was kept at 150°C with 10°C/min heating ramp. After this period, temperature was kept at 150°C for 2 minutes. Finally, temperature was increased to 280 with 20°C/min heating ramp and then kept at 280°C for 49 minutes and chemical substances of the honey samples were identified by using standard Nist Libraries available in the data acquisition system of GC-MS.

RESULTS and DISCUSSION

After melissopalynological analysis were obtained 10 monofloral chestnut, 2 monofloral rhododendron and 3 mixed chestnut&rhododendron honeys (Table 1) (Figure 2 and 3).

According to Turkish Food Codex (2012) [16] and Codex Alimentarius Committee on Sugars (2001) [17] sum of the fructose and glucose should be not less than 60g/100g and ratio of fructose to glucose should be between 1.0% and 1.85% for chestnut honeys. White (1978) [18] reported that the ratio of Fructose and Glucose in honey varied from 1.0 to 1.2 and this ratio could also change depending on the nutrition the beekeeper has made during the spring. Manzanares et al. (2017) [19] with 42 chestnut honeys, they reported that F 37.5%-44.1%, G 23.2%-30.9%, F + G 63.3%-74.8% and F/G 1.29- 1.78. Also Can et al. (2015) [20] found the values respectively, F 38.44%, G 19.35%, F+G 57.79%, F/G 1.98%, at chestnut honey. In our results we found Fructose between 29.20% and 42.99%, Glucose between 18.25% and 31.15%, F+G between 49.18% and 73.02% and ratio of F/G between 1.30% and 1.80% at chestnut honey samples. It was found Fructose 36.91% and 37.45%, Glucose 26.52% and 28.86%, F+G between 63.43% and 66.30% and ratio of F/G 1.31% and 1.38% at rhododendron honey samples. In our study, it was determined Fructose between 31.03% and 38.79%, Glucose between 23.26% and 32.61%, F+G between 55.99% and 71.00% and ratio

of F/G 1.17% and 1.52% at chestnut&rhododendron honey samples (Table 2, Figure 4). Consequently, codex and other studies have been found to be in accordance with our results of sugar analysis.

GC-MS chemical substance analyses of honeys revealed alcohols, aldehydes, ketones, aliphatic acids and their esters, carboxylic acids and their esters and flavonoids (Table 3). Bonago et al. reported that, hydrocarbons (n-Heptacosane, n-nonacosane, n-tricosane, n-pentacosane, and n-hentriacontane, etc), carboxylic acid and esters, were present in the chestnut honeys as a result of the chemical analysis. The same authors reported that in their another studies with unifloral chestnut honey, honeys contained 50 kinds of volatile components [21,22]. In recent years researchers determined furan derivatives in higher amounts in chestnut honey [1,23-25]. Also we found furan derivatives in this study especially in chestnut honey samples (C1,C5,C6,C7,C8,C9).

Radovic et al., (2001) [25] found furfural, which is an aromatic aldehyde, before in acacia honey. It was also determined in lime and lavender honey by Cuevas-Glory et al., (2007) [26]. In this study we

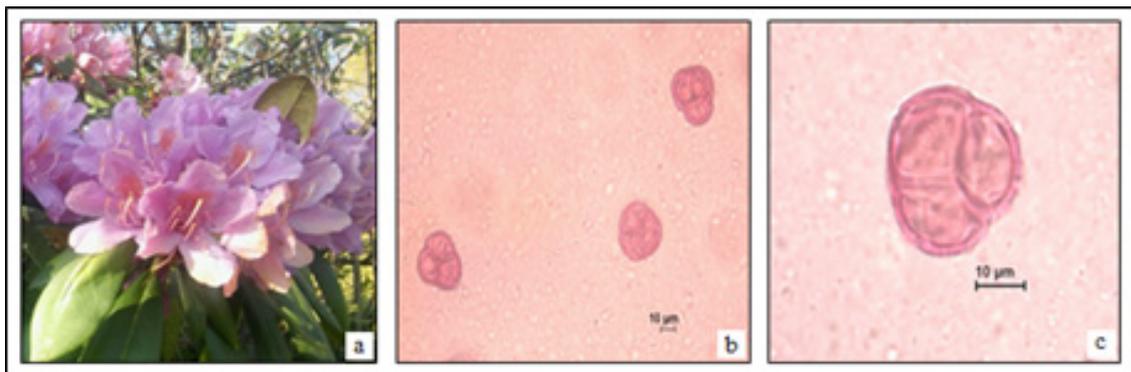


Figure 2. a. *Rhododendron ponticum*, b-c. pollen photos of *Rhododendron* spp.

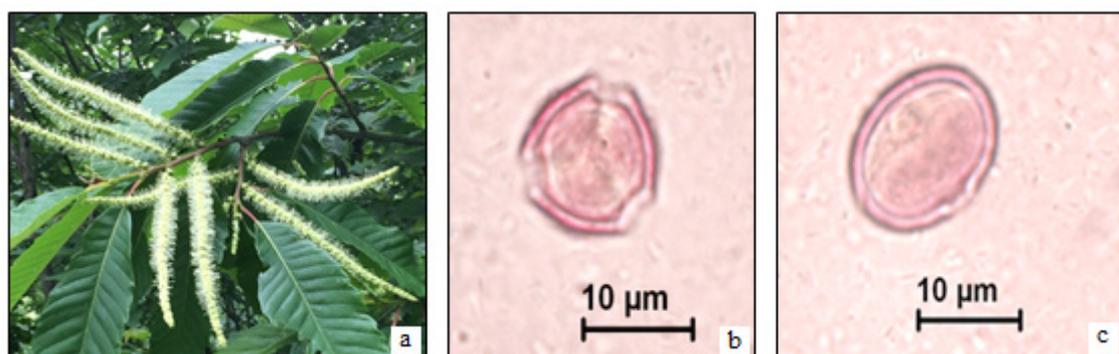


Figure 3. a. *Castanea sativa*, b-c. polar and equatorial view of *Castanea sativa* pollen.

Table 1. Melissopalynologic analysis of honey samples.

Honey sample	Total Pollen Number (TPN)	Predominant type of pollen (>45%)	Secondary pollen (15-45%)	Important Minor Pollen (3-15%)	Minor Pollen (<3%)	Region	Honey type
1	722419	<i>Castanea sativa</i> (97.5%)	-	-	<i>Rumex</i> sp. (1%) <i>Rosaceae</i> (1%) <i>Fabaceae</i> (0.5%)	Bartın	Chestnut
2	110844	<i>Castanea sativa</i> (97.7%)	-	-	<i>Brassicaceae</i> (1.7%) <i>Fabaceae</i> (0.4%)	Bartın	Chestnut
3	511271	<i>Castanea sativa</i> (97.5%)	-	-	<i>Rosaceae</i> (1.5%) <i>Asteraceae</i> (0.5%) <i>Rumex</i> sp. (0.5%)	Bartın	Chestnut
4	37626	<i>Castanea sativa</i> (91.5%)	-	<i>Ericaceae</i> (7.42%)	<i>Asteraceae</i> (1%)	Bartın	Chestnut
5	8733	<i>Castanea sativa</i> (90%)	-	<i>Ericaceae</i> (4.5%) <i>Rosaceae</i> (4.5%)	-	Bartın	Chestnut
6	292364	<i>Castanea sativa</i> (83.7%)	-	<i>Rosaceae</i> (8.86%)	<i>Ericaceae</i> (2.95%) <i>Lamiaceae</i> (2.46%) <i>Cistaceae</i> (1.97%)	Bartın	Chestnut
7	162796	<i>Castanea sativa</i> (92.5%)	-	-	<i>Rosaceae</i> (3%) <i>Lamiaceae</i> (2%) <i>Apiaceae</i> (1%) <i>Cistaceae</i> (0.5%) <i>Chenopodiaceae</i> (0.5%) <i>Poaceae</i> (0.5%)	Bartın	Chestnut

Table 1. Melissopalynologic analysis of honey samples (continue).

Honey sample	Total Pollen Number (TPN)	Predominant type of pollen (>45%)	Secondary pollen (15-45%)	Important Minor Pollen (3-15%)	Minor Pollen (<3%)	Region	Honey type	
8	63113	<i>Castanea sativa</i> (97%)	-	-	<i>Cistaceae</i> (1%)	Bartın	Chestnut	
					<i>Ericaceae</i> (0.5%)			
					<i>Rosaceae</i> (0.5%)			
					<i>Salix</i> sp. (0.5%)			
9	58975	<i>Castanea sativa</i> (97.5%)	-	-	<i>Cistaceae</i> (1.5%)	İstanbul	Chestnut	
					<i>Ericaceae</i> (1%)			
10	398568	<i>Castanea sativa</i> (98.5%)	-	-	<i>Rosaceae</i> (0.5%)	Kastamonu	Chestnut	
					<i>Apiaceae</i> (0.5%)			
					<i>Rumex</i> sp. (0.5%)			
11	4778	<i>Ericaceae</i> (50%)	<i>Castanea sativa</i> (38.8%)	<i>Poaceae</i> (11.1%)	-	Bartın	Rhododendron	
12	118658	<i>Ericaceae</i> (48.4%)	<i>Castanea sativa</i> (33.3%)	-	<i>Onobrychis</i> sp. (1.21%)	Bartın	Rhododendron	
			<i>Salix</i> sp. (15.15%)		<i>Cistaceae</i> (0.6%)			
13	14729	-	<i>Rosaceae</i> (22%)	<i>Fabaceae</i> (7%)	-	Düzce	Chestnut & Rhododendron	
			<i>Castanea sativa</i> (20%)					<i>Ericaceae</i> (7%)
			<i>Salix</i> sp. (18%)					<i>Brassicaceae</i> (7%)
			<i>Papaveraceae</i> (17%)					
14	888	<i>Castanea sativa</i> * (48%)						

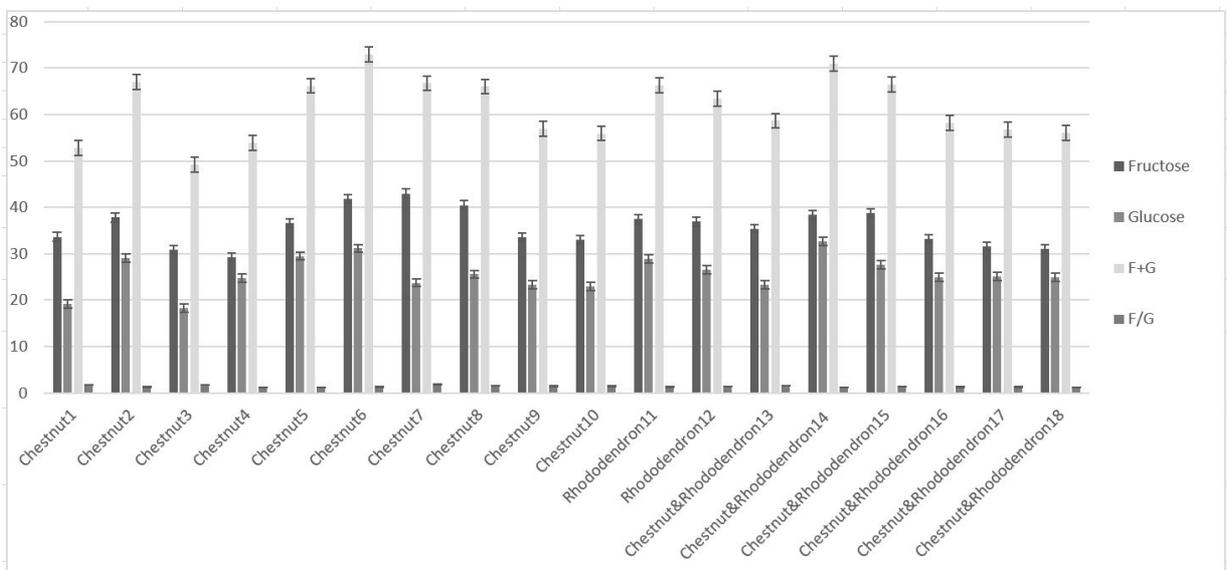
Table 1. Melissopalynologic analysis of honey samples (continue).

Honey sample	Total Pollen Number (TPN)	Predominant type of pollen (>45%)	Secondary pollen (15-45%)	Important Minor Pollen (3-15%)	Minor Pollen (<3%)	Region	Honey type
15	76039	<i>Castanea sativa</i> * (68%)	-	<i>Ericaceae</i> (14.5) <i>Asteraceae</i> (9.5)	<i>Fabaceae</i> (2.8) <i>Rosaceae</i> (2.5) <i>Apiaceae</i> (2.7)	Düzce	Chestnut& Rhododendron
16	1728	-	<i>Castanea sativa</i> (30%) <i>Ericaceae</i> (23.9%)	<i>Fabaceae</i> (14.1%) <i>Cistaceae</i> (14.1%) <i>Liliaceae</i> (9.8%)	<i>Centaurea</i> sp. (2.8%) <i>Rosaceae</i> (2.8%) <i>Hedysarum</i> sp. (1.4%) <i>Asteraceae</i> (1.4%) <i>Brassicaceae</i> (1.4%)	Düzce	Chestnut& Rhododendron
17	4142	<i>Castanea sativa</i> * (58%)	-	<i>Cistaceae</i> (9.6%) <i>Ericaceae</i> (8%) <i>Fabaceae</i> (8%) <i>Apiaceae</i> (5.6%) <i>Salix</i> sp. (4.3%) <i>Rosaceae</i> (4%)	<i>Campanulaceae</i> (1.6%) <i>Onobrychis</i> sp. (0.8%)	Düzce	Chestnut& Rhododendron
18	5186	<i>Castanea sativa</i> * (52.7%)	<i>Rosaceae</i> (15.2%) <i>Salix</i> sp. (20.8%)	<i>Ericaceae</i> (4.2%) <i>Fabaceae</i> (4.1%)	<i>Liliaceae</i> (1.3%) <i>Poaceae</i> (1.3%)	Düzce	Chestnut& Rhododendron

* Chestnut honey, a content of 70-90% of *Castanea* pollen is required to classify honey as unifloral.

Table 2. HPLC sugar analysis results of honey samples.

Honey Sample	Honey type	Fructose (%)	Glucose (%)	F+G (%)	F/G (%)
C1	Chestnut	33.64±2.87	19.14±2.37	52.78±4.54	1.76±0.19
C2	Chestnut	37.81±2.76	29.12±5.22	66.96±7.56	1.30±0.15
C3	Chestnut	30.92±3.95	18.25±2.63	49.18±6.36	1.71±0.09
C4	Chestnut	29.20±2.94	24.71±4.76	53.93±7.37	1.21±0.14
C5	Chestnut	36.68±0.80	29.52±3.02	66.20±3.32	1.24±0.13
C6	Chestnut	41.86±1.33	31.15±3.72	73.02±5.04	1.30±0.12
C7	Chestnut	42.99±2.20	23.76±1.77	66.75±3.68	1.80±0.09
C8	Chestnut	40.47±4.62	25.57±5.75	66.04±10.31	1.6±1.18
C9	Chestnut	33.62±4.48	23.38±4.44	57.00±0.65	1.49±0.42
C10	Chestnut	33.04±3.27	22.90±3.24	55.94±4.52	1.46±0.27
R11	Rhododendron	37.45±2.05	28.86±4.22	66.30±2.53	1.31±0.25
R12	Rhododendron	36.91±5.09	26.52±1.66	63.43±5.76	1.38±0.20
CR13	Chestnut&Rhododendron	35.41±0.83	23.26±0.86	58.67±1.19	1.52±0.07
CR14	Chestnut&Rhododendron	38.39±3.44	32.61±1.49	71.00±4.08	1.17±0.10
CR15	Chestnut&Rhododendron	38.79±1.12	27.62±1.90	66.41±1.66	1.40±0.12
CR16	Chestnut&Rhododendron	33.23±0.41	25.00±0.86	58.23±1.19	1.32±0.04
CR17	Chestnut&Rhododendron	31.56±1.07	25.13±0.68	56.7±1.17	1.25±0.05
CR18	Chestnut&Rhododendron	31.03±0.85	24.96±1.07	55.99±1.83	1.24±0.03

**Figure 4.** HPLC sugar analysis results of honey samples.

found furfural in seven samples of the ten chestnut honeys (C1,C2,C3,C5,C6,C7,C8) (Table 3).

Flavanoids are important compounds because of their antioxidant effects. Flavanones are one of the most important groups of the flavonoids. Yu et al., 2013 and Özkök et al., 2016 [27,28] determined 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) flavanones which is a strong antioxidant in the pine honey samples. Also we found DDMP in the C6, R12 and CR13 samples (Table 3).

In this study, the chemical composition of chestnut and rhododendron honeys and their sugar content were investigated and it was determined that they could show changes depending on the botanical origins of honey.

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