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# HIGHLIGHTING THE MELISSOPALYNOLOGICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF AYDER-RİZE (TURKEY)

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ABSTRACT. The aim of this research is characterizing the honey produced in Ayder/Rize-Turkey. In this context 20 honey samples were collected from Ayder in 2018. The melissopalynological and physicochemical analysis of the honey samples were done by this research. According to the melissopalynological analysis 40 taxa belong to the 22 plant families were identified as botanical sources of the investigated honey samples. Nine of the investigated samples (sample no 1-9) were evaluated as monofloral and the others as multifloral honeys. As a result of melissopalynological analysis, while pollen belong to the Castanea sativa taxon were found in dominant ratios in some of the investigated samples, the pollen belongs to the Castanea sativa, Trifolium repens, Lotus corniculatus, Coronilla orientalis taxa were found as secondary in some other honey samples. Total pollen number in 10 gram honey (TPN 10) values of the samples were found between 7 732 and 167 147 by melissopalynological analysis. The first step of the physicochemical analysis was the moisture analysis and the values found between 15,8% and 18,8% (mean value:7,01±0,98 %). The fructose/glucose analysis were done by High Performance Liquid Chromatography (HPLC) and the values for fructose found as: 26,43-35,57g/100g (mean: 31,39±2,41 g/100g), glucose values as; 20,11-30,58 g/100g (mean: 26,83±2,16 g/100g). Also fructose/glucose ratio was found as 1,03-1,34 (mean: 1,17±0,10). Hydroxymethylfurfural (HMF) and total phenolic acids analysis were done also by HPLC. The results for HMF analysis is between 0,7-11,31 ppm (mean:  $3,8\pm2,6$  ppm), for total phenolic acids the value are found between 88,92±0,04 mgGAE/kg - 196,17±0,10 mgGAE/kg (mean: 121,98±0,1 mgGAE/kg). As a step of chemical analysis; the volatile compounds of the honey samples were determined by Gas Chromatography and Mass

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Spectrometry (GC-MS). The compounds belong to the aldehydes, aliphatic acids and esters, alcohols, hydrocarbons, carboxylic acids and their esters, ketones, terpenes, fatty acids and their esters groups were found as a result of GC-MS analysis. The compounds belong to the carboxylic acids and their esters, fatty acids and their esters identified in higher ratios compare to the other compounds. Since, there is little detailed published information about the quality and properties of Ayder-Rize honey, the current study aims to characterize the honey belong to the this region.

#### 1. INTRODUCTION

Honey is a natural product that is produced by honey bees, especially by the species of *Apis mellifera*. Two types of honey is present: one kind as blossom honey comes from nectars of flowers and the second kind as honeydew honey (forest honey) is a type of honey made from honeydew secreted by plant-sucking insects such as aphids [1].

The chemical composition of honey is variable, owing to the numerous parameters. Its constituents are carbohydrates, water, organic acids, enzymes, amino acids, pigments, pollen and wax; some are added by the bees and some of them are sourced from the plants [2].

Sugars are the major components of honey. It consists mostly glucose and fructose [2]. Honeydew honeys have lower contents of glucose and fructose while have higher levels of oligosaccharides [3].

In many countries, honey is considered more as a medicine or special tonic, rather than as a food. Honey has medicinal properties that are acknowledged increasingly by modern medicine. Besides, honey is used as a source of sugars for making honey wines and beers, and in the manufacture of many secondary products: breakfast cereals, bakery goods, and a multitude of other value-added products. It is also, applied to wounds, burns, ulcers and promotes faster healing [4].

As is seen from the literature; there are so many published scientific researches about Turkish honey. The researches mainly based on characterization of honey on the basis of region or qualified the honey types according to their melissopalynological and physicochemical features. The studies in Turkey that contains melissopalynological analysis of honey samples began with Quistani [5-11].

Nowadays, the researches are mostly comprised the physicochemical analysis of honey samples collected from different regions of Turkey. Can et al. (2015) investigated 62 Turkish honey samples (11 unifloral honeys; chestnut, heather, chaste tree, *Rhododendron*, common eryngo, lavender, Jerusalem tea, *Astragalus*, clover and *Acacia*, two different honeydew honeys; lime and oak and seven different multifloral honeys) on the basis of physico-chemical and biochemical characteristics. They mentioned that physico-chemical and biological characteristics of honeys are closely related to their floral sources [12].

Kıvrak et al. (2017) investigated the 54 honey samples from eighteen different locations of Turkey (cedar from Konya, eucalyptus from Muğla, multifloral from Hakkari, *Rhododendron* from Kastamonu, *Vitex* from Aydın, carob from Muğla, clover from Diyarbakır, pine and heather from Muğla, sunflower from Konya, citrus from Antalya, *Sideritis* and thyme from Muğla, chestnut from Düzce, *Acacia* from Burdur, lavender from Isparta, cotton from Adana, linden from Artvin) [13].

Derebaşı et al. (2014) searched characteristics of honey samples collected from different cities of Black Sea Region of Turkey. Owing to the physicochemical results, they mentioned that Black Sea Region honeys indicate a good quality level, adequate processing, good maturity and freshness [14].

Malkoç et al. (2019) evaluated the honey samples collected from Anzer-Rize according to the melissopalynological analysis, total phenolic contents (TPC), total flavonoid contents (TFC), and total antioxidant activities [15].

Kanbur et al. (2021) searched the physicochemical parameter changes, aroma, melissopalynological properties, and heavy metal content of honey produced from different types of flora (chestnut and highland) in the Senoz Valley from Rize [17].

Despite so many scientific researches are existing about Turkish honey and especially produced in Anzer plateu - Black Sea Region of Turkey, there are little scientific publications about honey produced in Ayder plateu of Rize-Turkey [10,14,15,16,17].

The aim of this study was characterizing the honey samples collected from Ayder plateu of Rize-Turkey according to their botanical sources and physochemical characteristics. Ayder plateu has an important role for Turkish beekeeping owing to its virgin nature, floral circumstances and climatical conditions. Since there is little detailed data is available about the honey of Ayder plateu, the results will be a data source for the region.

# 2. MATERIALS AND METHODS

### 2.1. Honey samples

Honey samples were collected from Ayder-Rize that is located in the East of Black Sea Region. Twenty honey samples were collected between August- September of 2018 from different localities (Köy location-sample no 1-5, Kedmeç locationsample no 6-10, Galer location - sample no 11-15, Yayla location- sample no 16-20) of Ayder plateu/ Rize (Black Sea Region of Turkey) (Figure 1).



FIGURE 1. The map of Ayder plateu (http://gezilecekyerler.com/camlihemsin/)

# 2.2. Melissopalynological analysis

Preparation of honey samples for qualitative and quantitative mellisopalynological analysis was performed by Louveaux et al. (1978) [18]. The total pollen number (TPN) of all samples was calculated according to the method described by Moar (1985) [19]. The honey samples (10g) were classified according to total pollen number (TPN) as Group I: TPN<20000; Group II: 20000<TPN<100000; Group III: 100000<TPN<500000; Group IV: 500000<TPN<1000000 and Group V: TPN>1000000 [19]. 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%) [20].

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### 2.3. Physicochemical characterization of honey

### Moisture Measurement

Moisture analyses were done by a portable refractometer and determined as % (w/v) ratio.

# **GC-MS** Analysis

A GC 6890 N instrument from Agilent (Palo Alto, CA, USA) coupled with a mass detector (MS5973;Agilent) was used for the analysis of honey samples. Organic compounds in honey samples were identified in Wiley's NIST Mass Spectral Library, if they obtained comparison scores were higher than 95%.

#### Sugar Analysis

Sugar (Fructose/glucose) content was determined according to the harmonised methods of international honey commission's suggestions (2009). The samples were analysed by HPLC (Agilent Technologies, USA) with RID detector (Agilent Technologies, USA) and Zorbax (4.6x250mm, 5-Micron) carbohydrate column (Agilent Technologies, USA) [21].

# HMF Analysis

HMF content were analysed by HPLC. It was determined according to the harmonised methods of international honey commission's suggestions (2009). The samples analysed by HPLC (Agilent Technologies, USA) with UV detector Agilent Technologies, USA) and C18-reversed phase column (Agilent Technologies, USA) [21].

#### **Total Phenolic Compound Estimation**

Total phenolic compound of honey extracts estimated according to the Folin-Ciocalteu method described by Slinkard and Singleton (1977). The absorbances of samples were measured at 760 nm with UV/VIS spectrophotometer [22].

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# 3. RESULTS AND DISCUSSION

### 3.1 Melissopalynological analysis results

By palynological analysis of the investigated honey samples the pollen of taxa belong to the Apiaceae, Asteraceae, Berberidaceae, Betulaceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cistaceae, Cyperaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Rubiaceae and Salicaceae families were identified (Table 1).

Nine of the 20 samples were found as chesnut honey and the other 11 samples were evaluated as multifloral honey. With regard to the results, all investigated five samples of Köy location were detected as monofloral–chesnut honey. Five samples were collected from Kedmeç location and four of the samples qualified as monofloral-chesnut honey and the last one as multifloral. Five samples were collected from each Galer and Yayla locations and the samples were defined as multifloral.

Total pollen number in 10 gram honey (TPN 10) values of the samples were found between 7 732 and 167 147 by melissopalynological analysis (Table 1).

3.2 Physicochemical analysis results

Moisture analysis

The moisture values of the investigated samples were found between 15.8-18.8% (mean:17.01%) (Table 2). These results are suited to Codex Alimentarius (2001) and Turkish Food Codex, Honey Directive (2020) [23,24].

Sugar content analysis

According to the sugar analysis, the values for fructose found as: 26,43-35,57g/100g (mean:  $31,39\pm2,41$  g/100g), glucose values as; 20,11-30,58 g/100g (mean:  $26,83\pm2,16$  g/100g). Also the fructose/glucose ratios were found between 1.03-1.34 (mean:1.17) (Table 2). These results fit with the sugar values that is mentioned by Codex Alimentarius (2001) and Turkish Food Codex, Honey Directive (2020) [23,24].

Total phenolic contents

The total phenolic content of the samples are measured as minimum 88,92±0,04 mgGAE/kg and maximum 196,17±0,10 mgGAE/kg (mean: 121,98±0,1 mgGAE/kg) (Table 2).

#### HMF analysis

The HMF values are determined as minimum 0,7 ppm, maximum 11,31 ppm (mean:  $3,8\pm2,6$  ppm) (Table 2). Since, Codex Alimentarius (2001) and Turkish Food Codex, Honey Directive (2020) allow until 40 ppm, our findings are suitable in terms of HMF values.

# GC-MS analysis

The compounds belong to the aldehydes, aliphatic acids and esters, alcohols, hydrocarbons, carboxylic acids and their esters, ketones, terpenes, fatty acids and their ester groups were found as a result of GC-MS analysis. The compound belong to the carboxylic acids and their esters, fatty acids and their esters identified in higher ratios compare to the other compounds (Table 3).

Owing to the palynological analysis, taxa belong to the Apiaceae, Asteraceae, Berberidaceae, Betulaceae, Boraginaceae, Brassicaceae, Campanulaceae, Caryophyllaceae, Cyperaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Plantaginaceae, Poaceae, Polygonaceae, Ranunculaceae, Rosaceae, Rubiaceae and Salicaceae families were found as botanical sources of honey samples. These findings overlap with the flora of the study area. To confirm the palynological observations, plant samples were collected from the environments of the apiaries that samples collected during the field study. Our results are compatible with this plant list too.

Demir (2013) investigated 41 honey samples from Ayder-Rize and 21 samples from Kedmeç location. By this research, the pollen of taxa belong to the Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Ericaceae, Fabaceae, Fagaceae, Gentianaceae, Geraniaceae, Lauraceae, Malvaceae, Polygonaceae, Primulaceae, Ranunculaceae, Rosaceae, Salicaceae, Scrophulariaceae families were found in honey samples of Kedmeç location. These palynological findings are similiar with our results [25].

Also, Demir (2013) mentioned that all the samples are chesnut honey owing to dominant *Castanea sativa* pollen contents in honey samples. Similarly, we evaluated, four of five Kedmeç samples as chesnut honey [25].

Malkoç (2019) mentioned that honey samples from Anzer-Rize contains pollens from different family types at levels less than 45%, including; Lamiaceae, Fabaceae, Apiaceae, Rocaceae, Asteracea, Ericaceaa, and Liliaceae. Also mostly detected pollen defined as *Thymus, Rumex, Onobrychis, Cistus, Plantago, Ranunculus, Rhododendron, Myosotis* and *Geranium* [15]. In our research, the families contain determined pollens are very similiar with the results of Malkoç (2019) owing to the research area.

As a part of melissopalynological analysis, we calculated the "total pollen number in 10 gr honey (TPN 10) values are between 7732 and 167147 by melissopalynological analysis. Demir (2013) observed these values between 3 438-85 285 for honey samples collected from Kedmeç location. Our TPN 10 values for Kedmeç honeys are found between 11 046 - 36 247. Also Demir (2013) mentioned the identified taxa number vary from 4 to 14 for Kedmeç samples. We found this rate between 5-8 [25].

Moisture rate of the honey gives an idea about the harvesting of the honey. With regard to our results, the moisture values are between 15.8-18.8% (mean:17.01%). These results are suited to Codex Alimentarius (2001) and Turkish Food Codex, Honey Directive (2020). By previous researches; moisture of chesnut honey found as  $19.70\pm1.33\%$ , 16.21% [12, 13], and also Kedmeç-Ayder honey (evaluated as chesnut honey) as 16.6-20.6% [25]. Derebaşı et al. (2014) found the mean moisture value as  $16.6\pm0.12\%$  from different honey samples of Black Sea Region-Turkey and  $18.57\pm0.72\%$  for honey samples collected from Rize [14].

Hydroxymethylfurfural (HMF) content is a marker for freshness and overheating of honey (Book of honey 2009). According to Codex Alimentarius (2001) and Turkish Food Codex, Honey Directive (2020), permitted value for HMF is maximum 40 ppm. Our HMF results are in this limitation. By previous researches HMF values for chesnut honey were found as  $9.28\pm7.13$  ppm, 1.66ppm [12,13] and of honey samples collected from Rize as  $9.19\pm1.12$ ppm, as  $8.86\pm0.38$  ppm from different honey samples of Black Sea Region-Turkey [14].

Some previous researches about total phenolic contents of chesnut honey reflect the values as; 98.26±1.77 mgGAE/100g, 97,66 mgGAE/100g, 430±68 mgGAE/100g [12,13,17], also honey samples from Anzer as 240±52 mg GAE/100g [17]. Malkoç

et al. (2019) found the total mean phenolic content of Anzer honey as 26.92 mg GAE/100 g [15]. Hepsağ (2019) found the total phenolic content of the honey samples from Anzer plateau in Rize between 802.6- 1352.6  $\mu$ g GAE / g honey [26]. The total phenolic content of the samples are measured in our research as minimum 88,92±0,04 mgGAE/kg and maximum 196,17±0,10 mgGAE/kg (mean: 121,98±0,1 mgGAE/kg). It is clear that from the results our findings lower than the values from the previous researches.

The investigation about the invert sugar of honey samples collected from Black Sea Region of Turkey found for; Rize honey as  $72.874\pm2.12\%$ , fructose of chesnut honey as:  $38.44\pm2.72\%$ , glucose of chesnut honey as:  $19.35\pm3\%$  fructose of Kedmeç-Ayder honey as 16.31-65.28%, glucose of Kedmeç-Ayder honey as 17.23-64.47% [12,14,25].

The biological effects of the Black Sea Region honey also searched and Çakır et al. (2020) found that Anzer – Rize honey effected on *Staphylococcus aureus*, *Saccharomyces cerevisiae* and *Escherichia coli* [27].

# 4. CONCLUSION

As understood from the literature there are so many researches about characterization of honey collected from Black Sea Region of Turkey. While there are so many investigation about Anzer honey (Rize-Black Sea Region of Turkey), there are limited researches about honey collected from Ayder (Rize- Black Sea Region of Turkey). So our findings will be helpful for characterizing Ayder honey and light the way for the geographical indication surveys of Ayder honey.

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Author Contribution Statement Ö.G.Ç., A.Ö., Ç.Ö., and N.M performed the analysis, K.S., A.Ö., Ç.Ö., G.Z., N.M. carried out the field study, Ö.G.Ç. wrote the manuscript, All authors reviewed the manuscript

Declaration of Competing Interests The authors declare no conflict of interest.

23	22	21	20	19	18	17	16	15	14	13	12	=	10	9	8	7	6	IJ	4	3	2	1	Taxa no			
			Fabaceae		Ericaceae		Cyperaceae	Cistaceae	Caryophyllaceae	Campanulaceae		Brassicaceae		Boraginaceae	Betulaceae	Berberidaceae			Asteraceae			Apiaceae	Plant fanily			
Medicago spp.	Lotus corniculatus	Coronilla orientalis	Trifolium repens		Rhododendron spp.	Epigaea gaultherioides	Carex spp.			Campanula latifolia	Cardamine impatiens		Myosotis spp.	Cerinthe spp.	Betula spp.	Berberis spp.	Taraxacum spp.	Tanacetum sorbifolium	Petasites albus	Centaurea spp.	Anthemis spp.		Plant ta xon			
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Table 1. Melissopalynological analysis results of investigated honey samples

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	40	39	38	37	36	35	34	33	32	31	30	29	28	27	26	25	24	Taxa no					
TP	Tiliaceae	Salicaceae	Rubiaceae			Rosaceae	1	1	Ranunculaceae	Polygonaceae	Poaceae	Plantaginaceae			Lamiaceae		Fagaceae	Plant fanily					
V10 values	Tilia spp.	Salix spp.	Galium spp.		Rosa spp.	Rubus idaeus	Prunus spp.	Fragaria vesca	Ranunculus spp.	Rumex spp.		Plantago lanceolata	Thymus praecox	Teucrium spp.	Nepeta spp.	Ajuga orientalis	Castanea sativa	Plant ta xo n					
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HIGHLIGHTING THE MELISSOPALYNOLOGICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF AYDER-RİZE (TURKEY)

Honey number	Location	Moisture (%)	Fructose (g/100g)	Glucose (g/100g)	Fructose/ Glucose	HMF (ppm)	Total phenolic content (mgGAE/kg)
1	KÖY	16,3	35,57	26,54	1,34	6,5	178,91±0,32
2	KÖY	16,1	33,95	25,59	1,32	8,6	164,12±0,17
3	KÖY	16,5	33,61	26,42	1,27	5,3	179,63±0,20
4	KÖY	16,6	33,13	24,84	1,33	3,4	186,42±0,15
5	KÖY	16,6	31,92	26,08	1,22	3,7	196,17±0,10
6	KEDMEÇ	16,3	32,97	28,55	1,15	1,6	112,06±0,08
7	KEDMEÇ	16,2	30,00	24,97	1,20	1,5	$108,04{\pm}0,08$
8	KEDMEÇ	16,2	31,44	27,36	1,14	4,4	104,33±0,09
9	KEDMEÇ	15,8	28,86	27,81	1,03	1,9	99,52±0,06
10	KEDMEÇ	16,0	31,09	29,71	1,04	1,6	106,93±0,02
11	GALER	18,7	26,43	20,11	1,31	11,31	$104,58{\pm}0,08$
12	GALER	18,3	30,03	27,25	1,10	0,7	$104,41{\pm}0,01$
13	GALER	18,8	29,18	26,86	1,08	3,1	95,85±0,13
14	GALER	18,7	28,57	27,60	1,03	1,7	116,09±0,03
15	GALER	18,3	28,14	25,43	1,10	1,6	105,29±0,08
16	YAYLA	16,9	35,32	28,41	1,24	3,2	97,01±0,10
17	YAYLA	17,0	32,80	28,34	1,15	6,2	88,92±0,04
18	YAYLA	17,0	32,16	26,96	1,19	4,4	94,35±0,02
19	YAYLA	16,8	31,02	27,19	1,14	3,1	93,44±0,05
20	YAYLA	17,1	31,66	30,58	1,03	1,7	103,55±0,19
	Mean value	17,01±0,98	31,39±2,41	26,83±2,16	1,17±0,10	3,8±2,6	121,98±0,1

TABLE 2. Physicochemical analysis results of honey samples

# HIGHLIGHTING THE MELISSOPALYNOLOGICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF AYDER-RIZE (TURKEY)

Fatty acids	Terpenes	Ketones	Carboxylic acids and their esters	Hydrocarbons	Alcohols	Aliphatic acids and esters	Aldehydes	Chemical compounds
8.99	0.72	6.9	. 22.24	1.47	5.02	8.89	5.85	1
3.22	0.44	3.25	21.88	1.2	3.57	3.53	5.73	2
8.78	4.36	6.22	19.52	4.51	6.86	1.77	5.63	3
13.51	0	7.21	25.57	0.17	6.7	10.34	5.53	4
16.76	0.85	11.95	19.53	2.27	3.32	3.52	4.4	5
19.12	1.8	8.18	25.34	6.28	3.58	3.56	7.03	6
11.32	2.61	4.02	17.34	0.26	2.19	3.95	3.31	7
15.24	1.79	0.91	23.22	0.12	5.21	5.6	7.26	*
20.03	2.23	8.73	33.27	1.15	4.79	4.4	2.49	9
13	1.92	7.09	22.28	2.09	2.96	1.46	1.68	10
33.75	3.91	5.51	11.25	3.43	5.91	9.17	5.33	Ξ
7.2	0.92	31	17.06	2.05	7.74	0.09	2.72	12
13.91	2.16	4.98	17.98	0.19	3.32	4.21	2.63	13
16.75	0.88	8.46	26.59	4.74	6.89	2.87	3.68	14
29.46	2.34	8.69	16.29	ı	0.25	6.85	7.1	15
9.67	6.52	6.16	40.13	0.13	4.5	5.63	6.43	16
15.49	2.04	9.58	14.34	0.28	7.46	3.99	2.43	17
11.73	1.91	6.62	18.72	2.64	3.45	3.21	5.4	18
10.34	2.21	29.23	20.14	0.2	2.1	3.65	1.52	19
12.39		10.15	24.85	0.32	3.32	2.85	0.98	20

TABLE 3. GC-MS analysis results of investigated honey samples (% ratio)

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