Some Physicochemical Characteristics of Denizli Thyme (*Origanum onites*) Honey

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Abstract: In this study, the number of beekeepers staying within the borders of Denizli province, which is one of the important accommodation centers of wandering beekeepers with its large thyme fields, and their accommodation places were determined and some information about thyme honey was obtained. Especially in Gözler, Uzunpınar, Belenardıç and Güzelpınar neighborhoods of Pamukkale district and Yağcılar neighborhoods of Güney district, thyme fields are dense and approximately 24 thousand bee hives stayed in 2021. Some physicochemical analyzes were carried out, for example, 9 honeys in 2021 and 6 honeys in 2022, which were collected in July when the Denizli thyme honey produced in these areas was harvested. First, pollen analysis was made in honey samples, and it was determined that it was thyme honey. Then, proline, diastase activity (DA), moisture, electrical conductivity (EC), pH, free acidity (FA), HMF, fructose (F), glucose (G), total phenolic content (TPC) was determined in 15 honey samples. Average amount of proline in thyme honey samples in 2021 and 2022, respectively; 663.5 and 708.9 mg/kg, DA 10.9 and 11.75; humidity rates 17.1% and 17.3%; EC 560 and 540 µS cm-1 ; pH 3.8 and 3.8; FA; 26.4 and 28.8 meq.kg-1 ; HMF, 9.5 and 12.4 mg/kg; F+G 69.6% and 69%; F/G was determined as 1.13 and 1.10, and the TPC was determined as 75.9 mg GAE/100g. In the future, it will be possible to reveal the descriptive features of Denizli thyme honey from other honeys by conducting more comprehensive and advanced analyzes.

Keywords: Honey bee, thyme, smell, total phenolic content, HMF, proline

Denizli Kekik (*Origanum onites*) Balının Bazı Fiziko-Kimyasal Özellikleri

Öz: *Bu çalışma ile, geniş kekik alanları ile gezginci arıcıların önemli konaklama merkezlerinden biri olan Denizli ili sınırları içinde konaklayan arıcıların sayısı ve konaklama yerleri belirlenmiş ve kekik balına ait bazı bilgiler elde edilmiştir. Özellikle Pamukkale ilçesine bağlı Gözler, Uzunpınar, Belenardıç ve Güzelpınar mahalleleri ile Güney ilçesine bağlı Yağcılar mahallelerinde kekik alanları yoğunlukta olup yaklaşık olarak 2021 yılında 24 bin arı kolonisi konaklamıştır. Bu alanlarda üretilen Denizli kekik balı hasadının yapıldığı Temmuz ayında toplanan, 2021 yılında 9 adet, 2022 yılında da 6 adet bal örneğinde bazı fizikokimyasal analizler yapılmıştır. Bal örneklerinde öncelikle polen analizi yapılmış ve kekik balı olduğu tespit edilmiştir. Daha sonra 15 bal örneğinde prolin, diastaz aktivitesi, nem, elektiriksel iletkenlik, pH, serbest asitlik, HMF, fruktoz, glukoz, toplam fenolik madde belirlenmiştir. Kekik balı örneklerinde ortalama prolin miktarı 2021 ve 2022 yılı sırasıyla; 663.5 ve 708.9 mg/kg, diastaz aktivitesi 10.9 ve 11.75; nem oranları %17.1 ve %17.3; EC 560 ve 540 µS cm^{-1} ; pH 3.8 ve 3.8; FA; 26.4 ve 28.8 meq.kg^{-1} ; HMF, 9.5 ve 12.4 mg/kg; F+G %69.6 ve %69; F/G 1.13 ve 1.10 olarak toplam fenolik madde 75.9 mg GAE/100g olarak tespit edilmiştir. Gelecekte, daha kapsamlı ve ileri tekniklerin uygulandığı analizlerin yapılması ile, Denizli kekik balını diğer ballardan ayırıcı özelliklerini ortaya koymak mümkün olabilecektir.*

Anahtar Kelimeler: *Bal arısı, kekik, koku, toplam fenolik içeriği, HMF, prolin*

INTRODUCTION

Although the priority purposes are different, beekeeping, it is considered important by both developed welfare societies and developing societies. In developed countries, beekeeping is an input of vegetative production primarily because of the contribution of bees to pollination, in addition to being a bee products production activity. Being not dependent on soil, having low investment and operating costs, providing all kinds of materials and equipment from domestic sources, using less labor compared to other branches of agriculture, being easily stored and being sold at a value price, beekeeping provides employment, income and income to the rural population in developing countries. It is accepted as a means of providing healthy nutrition opportunities (Karacaoğlu et al., 2020).

According to TUIK 2020 data, there are 8 179 085 colonies in 82 862 registered enterprises in our country. 104 076 tons of honey were produced from approximately 8 million colonies. Aegean Region, which has almost 20% colony existence and 13% honey production in our country, is one of the important accommodation regions. Aegean Region has an important place in beekeeping in Turkey due to honey production and the presence of colonies. Considering its position in the country beekeeping (colony flow from outside the region), its weight in the country beekeeping increases even more (Karacaoğlu and Uçak Koç, 2007). According to TUIK's 2020 data, the province with the highest honey production in the Aegean Region is Muğla (900 583 colonies and 6103 tons honey). Muğla is followed by Aydın (257 738 colonies and 3643 tons honey) and İzmir (273 949 colonies and 1493 tons honey). Denizli province, on the other hand,

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ranks 5th with 766 tons of honey production potential in the Aegean Region.

While Denizli is located in the Aegean Region, the climate of the Aegean Region is completely invisible. Since it is at the gateway from the coastal areas to the interior regions, the climate of the interior regions is felt, albeit slightly. In the province, in general, summers are hot and dry, and winters are warm and rainy. It allows species such as alder, linden, hazelnut, chestnut, willow, fern, which are the vegetation types of the Black Sea Region, to grow in the region. The local tree forests of the region, on the other hand, form vertical stratification depending on the altitude. The vertical spread, which starts with red pine, leaves its place to black pine at 850 meters, and reaches the alpine pine border with Taurus cedar and then various juniper species at high altitudes. From here, meadows begin and forest cover ends.

The thyme planting areas started by the Provincial Directorate of Agriculture between 1998 and 2000, while it was 2000 decares at the beginning, reached a production level of 21 324 tons in an area of 172 461 da, according to the data of 2020, as a result of the interest of the regional farmers. Denizli alone has 93.4% of the thyme cultivation areas in Turkey and meets 89.3% of the production. Thus, Denizli is an important province in terms of thyme production in our country and the world. The origin of Denizli thyme is İzmir ball thyme. As a result of Denizli-Pamukkale Municipality's application for "Denizli Thyme" Geographical indication on January 27, 2020, it was registered as "Denizli Thyme" by the Turkish Patent and Trademark Office on April 9, 2021.

Denizli is one of the important bee accommodation centers of nomadic beekeepers thanks to its large thyme areas. Especially in Gözler, Uzunpınar, Belenardıç and Güzelpınar neighborhoods of Pamukkale district and Yağcılar neighborhoods of Güney district, thyme fields are concentrated. The fact that Denizli came to the fore in thyme production attracted the attention of beekeepers in local and surrounding cities, and with an increasing colony density over time, they came to these regions and started to produce thyme honey. Although the accommodation time varies according to the season, it can start like the end of April and May 15 and continue until the end of August as you go from low to high, which is usually the flowering period of thyme.

In 2021, a total of 24 913 colonies sheltered in Gözler, Akçapınar, Belenardıç, Uzunpınar and Güzelpınar neighborhoods of Pamukkale district of Denizli province. The most dense bee colonies are in Gözler district (8730 colonies, 35%), followed by Akçapınar (5088 colonies, 20.4%), Belenardıç (4951 colonies, 19.9%), Uzunpınar (3734 colonies, 15%) and Güzelpınar (2410 colonies, 9.7%) (Anonymous, 2021).

Thanks to its simple sugars, honey has become one of the most important energy sources of human beings as a valuable carbohydrate, and it has been accepted as a sweetener in ancient cuisine (Andre, 1961). Thanks to the carbohydrates (glucose and fructose), protein, organic acids, mineral salts, vitamins and enzymes it contains, honey was one of the most basic substances used in medicine and pharmacy in ancient times. Honey is one of the basic materials used in dessert and bread making, and it is frequently used in the preservation of fresh vegetables, fruits and meats without spoiling thanks to its bacteriostatic and bactericidal properties, and in the production of alcoholic beverages and vinegar thanks to its fermenter feature (Balandier, 1993).

Ancient sources, epigraphic and archaeological artifacts give us information about beekeeping, honey and production areas in a very wide geography in the Mediterranean basin. The most sought after honeys are produced in the Aegean basin. Honey obtained from thyme grown on Hymetto mountain in the Attica region of continental Greece was accepted as the best in the world in ancient times (Lenger, 2011). In the process from ancient times to the present, bees and products obtained from bees have always held an important place for human beings. Products such as honey, pollen, propolis, bee venom and beeswax obtained from bees have been used for years and stil maintain their importance and are used today as on the first day.

In this study, pollen determination, some routine chemical parameters and total phenolic compounds were determined in honey samples taken from beekeepers staying in regions of Denizli with large thyme areas.

MATERIAL AND METHODS

The present study made use of 15 thyme honey samples from *Thymus subs*. collected in 5 neighborhoods provinces (Gözler, 5 honey samples; Akçapınar, 3 honey samples; Belenardıç, 3 honey samples; Uzunpınar, 2 honey samples ve Güzelpınar, 2 honey samples) of Pamukkale district of Denizli, between 2021 and 2022. The samples were taken directly from the beekeepers. All samples were stored at 4-5^{o}C. Analyses were done within a 3-month time period after harvesting.

Melisopalynological analysis was performed on honey samples (9 pieces) taken from beekeepe rs in 2021 and it was determined that the dominant pollen in all honey samples was thyme pollen. Melissoplaynological analysis of honey samples (Analysis method according to DIN 10 760) and other honey analysis (proline, diastase activity, moisture content, electrical conductivity, free acid, HMF, fructose, glucose, sucrose, maltose; analysis method according to IHC method) were made at Muğla Sıtkı Koçman University, Food Analysis Application and Research Center, also total phenolic content analysis in honeys were performed at ADU, Faculty of Arts and Sciences, according to the Folin-Ciocalteu method (Singleton and Rossi, 1965).

Statistical Analysis

In this study, the mean and standard deviations of the data on the physicochemical properties of honey were determined using the SPSS package program.

RESULTS AND DISCUSSION

The melissopalynological analysis results of 6 honey samples collected in 2022 are given in Table 1. According to Table 1, the dominant pollen isolated in 6 honey samples was determined as 58-62% *Thymus subs*., 13-19% *Vitex agnus*

castus. *Apiaceae, Astragalus subs., Rosa subs., Helianthus annuus L., Fabaceae* were determined between 2-4% in honey samples.

The physicochemical parameters of honey samples taken from the same region for two consecutive years (2021-2022) are presented in Tables 2,3,4 and 5.

Table 1. Melissopalynological analysis results in honey samples

	Pollen (Botanical and Geographical origin)	H1	H2	H3	H4	H5	H6
Dominant pollen isolated (>%15)	*Thymus subs.* (Lamiaceae)	%58	%60	%62	%60	%59	%61
	Vitex agnus castus (Verbenaceae)	%17	%15	%19	%14	%14	%13
Significant pollen isolated (>%1)	Apiaceae	%3	%3	%3	%2	%4	%3
	Astragalus subs. (Fabaceae)	%4	%3	-	%3	%3	-
	Rosa subs. (Rosaceae)	%3	%3	%3	%3	%3	%3
	Helianthus annuus L. (Asteraceae)	%2	%2	%2	%2	%2	%2
	Fabaceae	%2	%2	-	%2	-	-
	Zea mays L. (Poaceae)	%2	-	-	-	%2	-
	Asteraceae		-	%2	-	-	-
pollen isolated (≤%1)	Dipsaceae	%1	%1	%1			
	Lauraceae	%1	%1		%1		
	Boraginaceae		%1				
	Myrtaceae			%1		%1	
	Poaceae			%1			
	Lamiaceae					%1	
	Erica subs. (Ericaceae)	%1					%1
	Onobrychis subs. (Fabaceae)	%1			%1		

Table 2. Physicochemical parameters in honey samples (2021 year) * Standard deviation

Sample	Proline (mg/kg)	Diastase activity (DU/kg)	Moisture (%)	EC(µS cm^{-1})	pH
H1	565.0	6.5	16.6	690	3.6
H2	825.3	16.2	16.8	720	3.9
H3	458.4	18.3	19.0	560	4.0
H4	950.0	8.6	17.3	490	3.9
H5	550.0	18.1	16.9	360	3.1
H6	465.0	4.9	18.0	420	4.0
H7	971.0	9.2	16.8	490	4.2
H8	414.3	6.5	15.6	420	3.8
H9	772.9	10.2	16.4	930	3.9
Mean	**663.5**	**10.9**	**17.1**	**560**	**3.8**
*SD	218.2	5.21	0.98	180	0.35
Range	414.3-971	4.9-18.3	15.6-19.0	360-930	3.1-4.2

Proline is an essential amino acid that comes predominantly from the salivary secretions of *A. mellifera* during the conversion of nectar into honey (Manzanares et al., 2014). The proline content is considered a measure of honey's maturity and in some cases an indicator of sugar adulteration. In this study, the amount of proline in honey samples changed in the range of 414.3-971 mg/kg in the first year, on average 663.5 mg/kg and in the second year it was determined as 708.9±126.46 mg/kg in the range of 264.0-1212.9 mg/kg. The limit value determined by the Turkish Food Codex for Anonymous (2020), flower honey, was found to be higher than 300 mg/kg for all honey samples except for 1 honey sample in this study. According to Akgun et al. (2021) multifloral honey, chestnut, acacia and rhododendron honey reported the amount of proline as 692.67, 758.56, 357, 535 mg/kg, respectively. Ecem Bayram (2023) determined the proline content in honey obtained from Tokat and its surroundings was between 384.41 and 1271.56 mg/kg. Bouhlali et al (2019) found the amount of proline in thyme honey to be 877.41 mg/kg. According to Karatas et al. (2019) determined the proline amount as 1244.16 and 744.80 mg/kg in thyme honey samples taken from Denizli Tavas district (2 pieces honey samples). In this study and in some other studies, it is seen that the variation in the amount of proline in honey is high. The reason for the variation may indicate that the honey was harvested before it is ripe, or there may be other reasons.

Diastase activity is mostly among the parameters used to evaluate the freshness and/or overheating of honey (Can et al., 2015). In terms of diastase activity, the thyme honeys in this study were found to be between 4.9-18.3 and an average of 10.9 in 2021, and an average of 11.75 in the honey samples of 2022, in the range of 5.31-17.49. The Council Directives 2001/110/EC (European Union, 2001) do not authorize less than 8 Scade units. Kıvrak et al (2016) reported diastase activity as 22.17 in thyme honey produced in Datça. For example, citrus honey generally has low diastase activity and therefore the European Commission has set a different limit for this type of honey (Serra Bonvehi and Ventura Coll, 1995). Differences in diastase activity in honey may vary depending on nectar collection time, amount of nectar flow, and sugar content because high concentrated nectar flow leads to lower enzyme content (Khan et al, 2015; da Silva et al, 2016).

One of the important indicators of the freshness and heat treatment of honey is HMF (Hydroxymethyl furfural). In this study, the HMF values of honey samples were found to be 4.1-20.6 mg/kg on average 9.5 mg/kg in 2021, and 12.42 mg/kg on average in the range of 5.48-26.31 in 2022 (Tables 3 and 5). Özkök et al. (2010) determined the highest amount of HMF in different flower and pine honeys in thyme honey (23.97±22.91) and chestnut honey (22.38±17.55), while the amount of HMF in clover, citrus, sunflower, geven, cotton and pine honey was 3.6-6.8 mg/kg. they have determined. The mean HMF value obtained in this study was determined by Özkök et al. (2010) determined for thyme honey. The amount of HMF in honey samples without heat treatment in the south of Spain was determined in the range of 0.19-41.16 mg/kg by Serrano et al., (2007). Terrab et al. (2002) determined the amount of HMF between 3.8 and 48.4 mg/kg in different types of honey in Moracca, which has a climate that may cause an increase in HMF content. Ozkok et al. (2010) determined the amount of HMF in thyme (23.97±22.91) and chestnut honey (22.38±17.55) higher than some flower (clover, citrus, sunflower, locust, cotton) and pine honey (range 3.66-6.18). The mean HMF value determined in this study was lower than the value determined by Özkök et al. (2016) is higher than the values found.

Table 3. Physicochemical parameters in honey samples (2021 year)

Sample	Free Acid (meq.kg^{-1})	HMF mg/kg	F+G (g)	F/G (%)	TPCmg GAE/100 g
H1	20.3	16.5	70.3	1.01	76.8
H2	25.3	6.0	68.8	1.08	79.3
H3	36.3	20.6	71.1	1.03	73.9
H4	29.0	10.9	67.3	1.15	71.8
H5	34.6	13.3	74.3	1.18	74.8
H6	19.3	4.6	65.4	1.17	77.5
H7	18.0	5.8	74.1	1.17	78.7
H8	18.5	4.1	64.0	1.17	81.3
H9	36.7	4.2	71.3	1.20	69.8
Mean	**26.4**	**9.5**	**69.6**	**1.13**	**75.9**
*SD	7.90	6.1	3.6	0.07	3.72
Range	18-36.7	4.1-20.6	64-74.3	1.01-1.20	69.8-81.3

* Standard deviation

In this study, the moisture content of thyme honey samples produced in 2021 was between 15.6-19.0% and an average of 17.1%; In 2022, it was determined as 17.33% on average in the range of 15.88-19.08. The average moisture values obtained in this study were found to be higher than the value determined by Kıvrak et al (2016) (16.23%) and Terrab et al (2004) for thyme honey (16.3%).

In this study, electrical conductivity (540 and 560 µS cm-1) and and free acidity (26.4 and 28.8 meq.kg-1) values found for thyme honey were found to be compatible with the literature (Terrab et al., 2004; Kıvrak et al., 2016). The average pH value (3.8) found for thyme honey in this study was again for thyme honey by Terrab et al., (2004) (pH:4.2); and Kıvrak et al., (2016) (pH: 4.44).

In another study, the glucose and fructose ratios in a thyme (*Thymus spp*.) honey taken from Aydın were respectively; It was reported as 31.29±3.32%, 35.70±2.65% (Özkök et al., 2010.). The total fructose and glucose ratios (69.6 and 69) obtained in this study were determined by Özkök et al. (2010) is higher than that determined. Özkök et al. (2010) determined the total glucose-fructose ratio of 58-71% in different flower honeys such as clover, citrus, sunflower, chestnut, geven, cotton and pine honey. In terms of total glucose-fructose ratio, Oddo et al. (2004a) found rape, acacia, rosemary, honeydew, heather, chestnut, citrus, eucalyptus, sunflower and lavender honeys between 58-76%. The same researchers found the lowest glucose-fructose ratio in honeydew honey and the highest ratio in sunflower honey, and also reported that rape honey is richer in glucose (40%) than fructose.

Table 4. Physicochemical parameters in honey samples (2022 year)

Sample	Proline (mg/kg)	Diastase activity (DU/kg)	Moisture (%)	EC(µS cm^{-1})	pH
H1	264.0	5.31	17.0	710	4.4
H2	1212.9	17.49	19.1	380	3.6
H3	658.5	15.61	16.4	440	3.8
H4	587.9	5.60	18.6	710	3.8
H5	718.3	13.14	15.9	390	3.6
H6	811.5	13.39	16.9	600	3.5
H7	708.9	11.75	17.3	540	3.8
H8	309.76	5.13	1.26	160	0.33
H9	264.0-1212.9	5.31-17.49	15.9-19.1	380-710	3.5-4.4
Mean	**264.0**	**5.31**	**17.0**	**710**	**4.4**
*SD	1212.9	17.49	19.1	380	3.6
Range	658.5	15.61	16.4	440	3.8

* Standard deviation

Table 5. Physicochemical parameters in honey samples (2022 year)

Sample	Free Acid (meq.kg^{-1})	HMF mg/kg	F +G (g)	F/G (%)
H1	14.9	5.5	66.1	1.16
H2	34.1	26.3	70.1	1.02
H3	27.9	6.6	67.9	1.19
H4	25.6	6.3	71.1	1.05
H5	31.3	14.6	69.7	1.07
H6	39.1	16.3	69.0	1.11
Mean	**28.8**	**12.4**	**69.0**	**1.10**
*SD	8.3	8.08	1.8	0.066
Range	14.9-39.1	5.5-26.3	66.1-71.1	1.02-1.19

*Standart deviation

The fructose/glucose (F/G) ratio is a recommended ratio to explain the granulation of honey, because glucose is less water-soluble than fructose, and therefore appears to be the parameter that best predicts the crystallization tendency of honey (Laos et al., 2011). The F/G ratio in this study ranged from 1.01 to 1.20 F/G ratio of 1.14 and below indicates fast crystallization. A value above 1.58 indicates that honey does not have a tendency to crystallize (Venir et al., 2010) and a value of 1.3 indicates slow crystallization (Dobre et al., 2012). The amount and composition of phenolic substances in honey varies depending on the floral sources (Rababah et al., 2014). In this sense, phenolic components in the composition of honey are emphasized because they have an antioxidant effect due to their capacity to scavenge free radicals and because they are used as floral markers (Güzel and Bahçeci, 2019). In this study, the total phenolic content (TPC) of Denizli thyme honey varied between 69.8-81.3, and the TPC was determined as 75.9 mg GAE/100g (Table 3). Özkök et al. (2010) found the highest amount of TPC in citrus, sunflower, clover, cotton and chestnut honeys in chestnut honey (156.86±3.82 mg GAE/100 g) and the lowest in clover and cotton honey (38 mg/100 g). Kıvrak et al., (2016) determined the amount of TPC in thyme honey as 106.46 mgGAE/100g. Meda et al. (2005) determined the TPC substances in flower and honeydew honeys as 32-114 mgGAE/100g on average 74 mg. Karataş et al (2021) determined the TPC of thyme honey obtained from Denizli Tavas as 5.31±1.24 and 23.44±1.22 µg PEs/mg honey (The average TPC determined in this study (75.9 mgGAE/100g) was close to the phenolic content in sunflower (69 mg) and citrus (94 mg) honey. Karataş et al. (2021) determined TPC based on the values of pyrocatechol (µg PEs/mg honey). This is thought to be due to the fact that it is different from this and other studies (Meda et al., 2005; Özkök et al., 2010; Kıvrak et al., 2016).

CONCLUSION

It is known that there are more than 100 monofloral honey types in Europe (Oddo et al., 2004b). Thanks to its rich flora in Turkey, it is possible to produce a large number of monofloral honey. Sunflower, citrus, clover, chestnut, chasteberry, linden, heather, thyme, astragalus and pine honey are just some of them.

Within the borders of Denizli, according to the data of 2020, 172,461 da. *Thyme* cultivation is carried out in the area, and during the flowering period of thyme, wandering beekeepers stay with 24,913 active colonies in the Gözler, Uzunpınar, Belenardiç neighborhoods of Pamukkale district to produce thyme honey. The fact that thyme plant is a medicinal plant makes thyme honey more valuable than some other honeys, and the fact that it is a single plant honey (monofloral) increases this value. Due to the unique irritating smell of honey, it is usually consumed by mixing with other honeys. However, the fact that it is rich in total phenolic substances compared to other flower honeys makes this honey valuable. Özkök et al (2016) in their study on the antibacterial effect of Denizli thyme honey showed that honey has the potential to be effective against common causes of nosocomial infections, however, further research is required to confirm whether the application of honey is sufficient to kill target microorganisms in infected wounds without major local or systemic causes. indicated that they were needed. Studies have been carried out on thyme honey obtained from many different regions of our country, but studies on honey produced from thyme, also known as ball thyme and registered as Denizli thyme, are limited. This study is a preliminary study. For this reason, in future studies, more accurate information will be obtained with sensory and physicochemical analyzes to be made on samples collected from thyme honey produced under controlled conditions.

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