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PALYNOCHEMICAL QUALITY MONITORING OF HONEY SAMPLES IN AND AROUND ESKİŞEHİR PROVINCE, TURKEY

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Abstract

Honey is used in traditional medicine for a long time. Nectar which is the most important component of honey was produced by plants. In the present study, pollen and biochemical analysis of honey samples collected from different regions of Eskişehir were studied. Seventy taxa of pollens under 29 family and 41 genera in 41 honey samples were identified. Pollens were classified as dominant, secondary, minor and rare for frequency distribution. According to Wodehouse method, pollen spectra was determined. Palynochemical properties of honey samples in and around Eskişehir were deduced.

Introduction

Currently, there are approximately 375.000 plant species growing up naturally and about 500 of these species are plants with nectaries (Atkins 1946, Ioyrish 1974, Crane 1978, 1984). According to the 10 volume research of Davis (1965-1988), "Flora of Turkey and the East Aegean Islands", nearly 10022 plant species naturally grow in Turkey and about 3000 of these are endemic plants (Davis 1965, Davis *et al*. 1988). Of these species, approximately 450 types are of importance in terms of beekeeping. Turkey is one of the most suitable countries for beekeeping (Sorkun 2002). Pollen analysis carried out on honey is the most accurate and easiest method to identify plants with nectaries. Early studies from various parts of the world have demonstrated that the botanical and geographical origin of honey could be discovered through pollen analysis (Lieux 1972, Agwu and Akanbi 1985, Battesti and Goeury 1992). The first comprehensive melissopalynologic research in Turkey was carried out by Sorkun and İnceoğlu (1984) and a total of 162 types of pollen were identified from 94 honey samples gathered from Central Anatolia. Flowering plants with nectar have been identified and confirmed through pollen analyses carried out on honey samples gathered from 26 samples from Rize (Sorkun *et al*. 1989), 73 samples from various regions in Turkey (Sorkun and Doğan 1995, 1999), 28 samples from Anzer, Rize (Sorkun and Doğan 1985) and 24 samples from Konya (Kaplan and İnceoğlu 2002).

The research area of the study are the provinces, towns and villages of the city of Eskişehir, which is located in the northwest of the Central Anatolia and between 29° 58′ and 32° 04′ east longitudes, and 39° 06′ and 40° 09′ north latitudes. It is adjacent to Black Sea Region in the north, to Marmara in the northeast, and to Aegean Region in the west and southwest. Its geographical, climatic and floral features bear a resemblance to those of Black Sea, Central Anatolia and Aegean Regions. The north parts of the city are under the influence of climatic features of Black Sea and Marmara Regions. Due to its geographical location, the centre of Eskişehir has very-cold, semi-arid Mediterranean climate features. However, the province of Sarıcakaya has almost rainy Mediterranean climate.

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When the flora of Eskişehir territory is examined, it is observed that a total of 531 taxa belonging to 58 family and 268 species naturally spread all over the region. Approximately 10.31% of these taxa is endemic to Eskişehir territory. The families containing the most endemic taxa are Fabaceae and Asteraceae. When it is considered that the rate of endemism is about 30% all over Turkey, it could be stated that Eskişehir is rich in endemism. The Central Anatolian region has 253 endemic taxa although the research area has 115 endemic taxa that are members of 24 families. Some of these taxa are the plants that naturally grow only in Eskişehir (Tubives 2016). The highest number of types in the distribution of taxa to phytogeographical regions in Eskişehir territory belongs to Irano-Turanian types. Rapidly growing population in recent years, opening new residential areas without scientifically examining areas, waste gases from factories, and waste materials polluting water, soil and atmosphere also badly affect the flora. Besides, pesticides exterminate both endemic and non-endemic species, as well.

The quality of honey is positively affected and the commercial value of honey is increased through honey analysis studies. The increase in pollen and biochemical analyses carried out on honey will enable consumers and beekeepers get healthily informed about the field and will have honey produced in Turkey made known abroad.

Melissopalynologic analysis method has been made use of in the present study in order to identify plant and geographical origin of the honey samples gathered from Eskişehir territory (Maurizio and Hodges 1951, Louveaux *et al.* 1970). The reason for carrying out the study in Eskişehir is that the territory has an important potential in beekeeping, has a typical climate, flora and different plants with nectary and no comprehensive study has been implemented so far on the honey of the territory. Thus the present study was carried to identify both the quality of honey produced in Eskişehir by researching pollen ingredients and the plants that are the main source of pollen and nectar for the bees. Another purpose of the study has been to create the list of nectary plants in Eskişehir and to make a contribution to the list of nectary plants of Turkey.

Materials and Methods

Forty one honey samples gathered from Eskişehir territory were investigated within the study in July, August, September and October in 2007 and 2008 from the city centre and districts of Eskişehir (Fig. 1). Microscopic analyses were carried out on the samples. The regions from which the honey samples were gathered are shown in Table 1.

Because the study was aimed to be a comprehensive one, elaborate attention has been paid to gather many samples. While collecting the honey samples, the altitude of and the distance between the regions have been taken into consideration and the samples have been gathered from stationary hives.

The hives were opened by beekeepers and minimum 250 grams of honey was gathered from each hive. After being taken out, each of the samples were immediately put into sterile jars and capped and labelled properly. After having been taken to the laboratory for analyses, all the jars were kept in dry and dark cupboard at room temperature.

Within the area where the hives are located, the plants which bees may be interested in have been gathered, pressed and identified in the laboratory after having been properly dried. Reference pollen preparates were made from the pollens obtained from the flowers of these plants in compliance with Wodehouse method (Wodehouse 1959).

The method used for making the necessary preparates for pollen analysis is an international method used by the experts in apiculture institutes in eight European countries. In accordance with the Wodehouse method, minimum 4 pollen preparates have been prepared from each of the 41 honey samples (Wodehouse 1959, Erdtman 1969). These preparates have been examined via

Nikon Eclipse E400 microscope and immersion objective lens (x100) was used for identifying and taking micro-photographic shots of the pollens. During the examinations, all the area of 18x18 mm cover slips have been scanned and all the pollens within this area have been identified. In order to identify correctly these reference pollen preparates gathered from Eskişehir territory, the collection of pollen preparates from the Department of Biology of Gazi University were utilized.

Table 1. Locations list of honey samples.

Hive location	Order	Dominant pollen content
Center, Ömür	001	Vegetable, thyme, willow, wild flowers
Center, Tandır	002	Forrest plants
Çifteler Çatmapınar	003	*Heliotropium* ssp., thyme
Center, Karabayır	004	Garden flowers, thyme, willow, wild flowers
Mihalıççık, Ahurözü	005	Thyme, *Verbascum* ssp., *Quercus* ssp.
İnönü, Dutluca	006	Tyhme
İnönü, Kümbet Yeniköy	007	Wild flowers, thyme
İnönü, Kuzfındık	008	Thyme, clover
Center, A. Kartal	009	Witch grass, clover, trefoil
Center, Fevzi Çakmak	010	Witch grass, sunflower
Center, Muttalip	011	Vegetable, thyme, willow, wild flowers, sunflower
Center, Akpınar	012	*Salvia* ssp., *Hypericum* ssp., *Melissa* ssp., thyme
Center, Karaçoban	013	Wild flowers, thyme
Center, Karacahöyük	014	Sunflower, field flowers, vegetable, corn
Eğriöz Village	015	Thyme, upland flowers, fruits
Seyitgazi	016	Willow, clover, thyme, mixed flowers
Buldukpınar Village	017	*Verbascum* ssp., thyme, upland flowers
Günyüzü	018	Sunflower, wild flowers
City center of Eskişehir	019	wild flower, thyme, henbit, sunflower
Mahmudiye	020	Sunflower, upland flowers
Çifteler	021	Chestnut
Alaköy	022	Thyme, upland flowers
Center, Ömür	023	Thyme, *Verbascum* ssp., medick, upland flowers, willow, acacia, cedar
Karaçoban Village	024	Thyme, *Astragalus* ssp., upland flowers, sage
City center of Eskişehir	025	Thyme, pine, acacia, fruit flowers
Seyitgazi, Doğançayır	026	Clover, trefoil, vetch, sunflower, beet, wheat, barley, corn, daisy, *Lamium* ssp., *Verbascum* ssp., thyme
Center, Ömür	027	Thyme, *Verbascum* ssp., trefoil
Alpu	028	Sunflower, safflower, field flowers, clover
Yıldırım Farm	029	wild flower, fruit, vegetable, thyme
Meşelik forrest, Yenikent	030	Thyme, yellow and red clover, *Astragalus* ssp., almond, acacia, apple, pear, plum, *Quercus* ssp.
Alpu, Gündüzler	031	Sunflower, clovers
Mihalıççık, Dinek	032	Thyme, clover, *Verbascum* ssp., *Lamium* ssp.
Alpu	033	Thyme, upland flowers, *Verbascum* ssp.
Beylikova	034	Wild flowers, fruit, vegetable
Sulukaraağaç	035	Pine, cedar, daisy
Çifteler, Karaköprü	036	Wild flowers, fruit, vegetable
Sivrihisar, Kaymaz	037	Wild flowers, fruit, vegetable
Sivrihisar, Paşakadın Village	038	Wild flowers
Sivrihisar, Dümrek	039	Wild flowers, fruit, vegetable
Günyüzü, Atlas Village	040	Upland flowers, vegetable, pine, fruit trees
Sivrihisar, Kaymaz	041	Sunflower, upland flowers, *Verbascum* ssp.

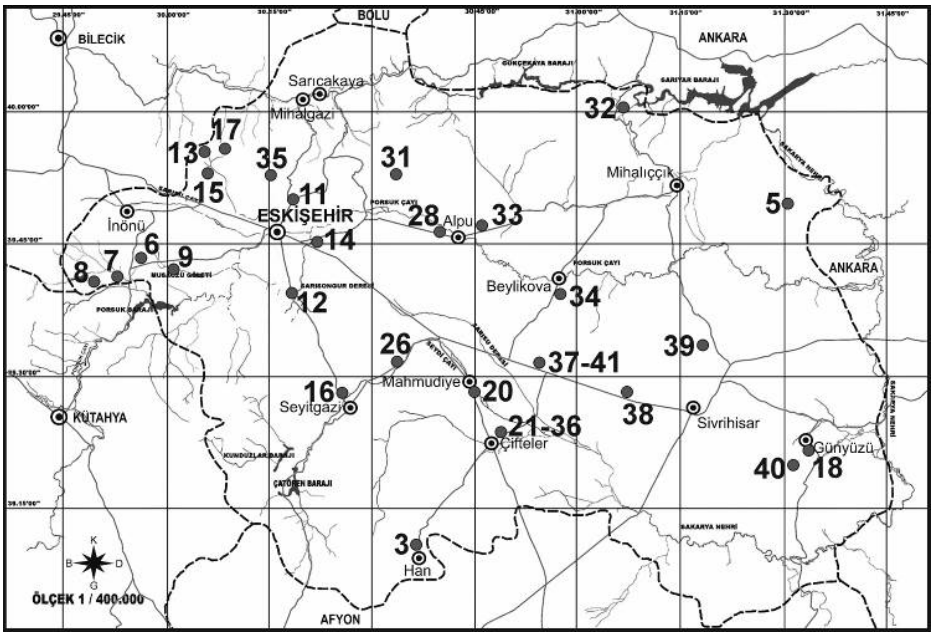

Fig. 1. Localities of investigated honey specimens from Eskişehir province.

For diagnostic purposes, each of the honey samples was filled into two test tubes and a total of 4 pollen preparates, two from each tube, were prepared. After the plant taxa to which the pollens in the preparates belongs were identified, a total of 200 pollens were counted in each of the preparates through x10 and x40 objective lenses. Then, the average pollen counts and percentages of the taxa and the contribution of these pollens in the taxa to the examined honey were determined. Besides, total pollen count (TPC) was identified. Fourteen honey samples to represent all honeys have additionally been analysed in terms of diastase, HMF, conductivity, proline, commercial glucose, acidity, moisture, purity, sugar components, and chemical properties. The chemical analyses of these 14 samples were carried out at Marmara Research Centre of TUBITAK (Gebze, Kocaeli) (Tables 2-4).

Results and Discussion

A total of 70 taxa were identified from 41 localities as a result of palynological analyses carried out on honey samples. A total of 70 pollen of the taxa, 29 of which are at the family level and 41 of which are at the species level, were identified from Eskişehir honey. Most of these pollens belong to Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, Fabaceae, Fagaceae, Lamiaceae, Rosaceae, Plantaginaceae, Poaceae, and Cistaceae families. The number of taxa types whose pollen was identified from the honey samples varied between 7 and 25. When the honey samples from Eskişehir territory were examined in terms of TPN-10 g, 0.73% of the samples was determined as poor in pollen, 1.21% as having normal pollen, 43.90% as rich in pollen and 36.58% was determined as very rich in pollen. 18 samples out of 41 were confirmed as unifloral honey since having only dominant or trace amount of pollen while the remaining 23 samples were identified as multifloral honey (Tables 2-3). It was determined that the honey of Eskişehir territory is multifloral in general.

Table 2. Pollen number, percentage of pollen, pollen spectrum and the total number of pollen in honey from Eskişehir province.

Hive location	Pollen number (%)	Pollen (%)	Pollen spectrum	Total no. of pollen (TPS-10 g)	Hive location	Pollen number (%)	Pollen (%)	Pollen spectrum	Total no. of pollen (TPS-10 g)
001	99.4	49.15	Dominant	12127.18	022	40.2	36.94	Secondary	7328.31
002	100	64.5	Dominant	22803.63	023	16.6	25.69	Secondary	4337.44
003	12	20.16	Secondary	3202.21	024	15.8	34.19	Secondary	6518.53
004	111	52	Dominant	13921.62	025	83	30.62	Secondary	5537
005	237	68.69	Dominant	27522.72	026	37.8	36.48	Secondary	7204.97
006	150	54.15	Dominant	14813.38	027	71.6	57.46	Dominant	16943.53
007	8	9.63	Minor	1337.81	028	17.4	30.31	Secondary	5455.77
008	2	2.9	Trace amount	385.90	029	6.4	17.29	Secondary	2606.12
009	3	6.1	Minor	817.97	030	117.8	74.55	Dominant	36752.42
010	18	30	Secondary	5375.14	031	41.4	38.54	Secondary	7867.25
011	7	17.07	Secondary	2582.17	032	64.4	46.19	Dominant	10769.39
012	27	24.77	Secondary	4180.66	033	170.4	69.21	Dominant	28194.68
013	15	13.15	Minor	1900.30	034	6.2	12.91	Minor	1860.29
014	7	15.21	Secondary	2251.12	035	8	14.13	Minor	2064.52
015	111	50.22	Dominant	12655.01	036	181.6	56.67	Dominant	16409.41
016	21	22.10	Secondary	3559.21	037	6.4	2.79	Trace amount	1791.71
017	19	30.64	Secondary	5541.81	038	27	22.39	Secondary	6047.03
018	29	29.59	Secondary	5271.27	039	150.8	54.26	Dominant	14480.30
019	10	25	Secondary	3135.5	040	152.4	56	Dominant	18203.81
020	121.4	49.18	Dominant	12103.32	041	2.6	2.9	Trace amount	501.68
021	129.2	61	Dominant	20054.78					

Table 3. The list of plant taxa determined as a result of palynological analysis performed on honey samples.

Number	Taxonomical group	Number	Taxonomical group	Number	Taxonomical group
1	+ Asteraceae	25	*Quercus* ssp.	48	*Erica* ssp.
2	*Centaurea* ssp.	26	*Eleagnus* ssp.	49	+ Rhamnaceae
3	*Artemisia* ssp.	27	+ Gentianaceae	50	+ Caprifoliaceae
4	*Taraxacum* ssp.	28	*Gentiana* ssp.	51	*Sambucus* ssp.
5	+ Fabaceae	29	*Ailanthus* ssp.	52	*Urtica* ssp.
6	*Hedysarum* ssp.	30	+ Rubiaceae	53	*Galium* ssp.
7	+ Brassicaceae	31	*Ulmus* ssp.	54	+ Caryophyllaceae
8	*Salix* ssp.	32	*Populus* ssp.	55	*Phaseolus* ssp.
9	*Fraxinus* ssp.	33	*Convolvulus* ssp.	56	*Echium* ssp.
10	+ Rosaceae	34	*Fagus* ssp.	57	*Fumana* ssp.
11	*Plantago* ssp.	35	+ Polygonaceae	58	+ Dipsacaceae
12	+ Apiaceae	36	*Laurus* ssp.	59	*Scabiosa* ssp.
13	+ Boraginaceae	37	*Platanus* ssp.	60	*Linaria* ssp.
14	+ Chenopodiaceae	38	*Juglans* ssp.	61	*Rumex* ssp.
15	*Castanea* ssp.	39	*Aesculus* ssp.	62	+ Oleaceae
16	+ Iridaceae	40	*Xanthium* ssp.	63	*Ligustrum* ssp.
17	+ Liliaceae	41	+ Cyperaceae	64	*Matthiola* ssp.
18	+ Poaceae	42	+ Cistaceae	65	*Wiedemannia* ssp.
19	*Cistus* ssp.	43	+ Pinaceae	66	+ Campanulaceae
20	+ Lamiaceae	44	*Cedrus* ssp.	67	+ Plantaginaceae
21	+ Scrophulariaceae	45	+ Euphorbiaceae	68	*Astragalus* ssp.
22	+ Ranunculaceae	46	*Mercurialis* ssp.	69	*Tilia* ssp.
23	+ Geraniaceae	47	+ Ericaceaea	70	Alnus ssp.
24	*Acer* ssp.				

+ Genus not determined.

Table 4. Chemical analysis of 14 honey samples.

Analysis	Results													
	1	3	5	15	17	20	22	23	26	28	32	33	34	41
Difference between protein and raw honey δ ^{13}C values in honey $^{(++)}$	-0.46	-0.30	0.10	0.25	0.12	0.18	0.29	-0.39	0.13	-0.70	0.27	-0.22	-0.22	-0.70
δ ^{13}C (honey)	-24.54	-24.49-	-25.10	-24.63	-25.12	-25.02	-25.42	-24.76	-25.01	-24.31	-25.87	-24.59	-24.04	-24.74
	-24.57	-24.45	-25.09	-24.64	-25.16	-25.02	-25.37	-24.68	-25.11	-24.37	-25.94	-24.58	-24.12	-24.73
δ ^{13}C (protein)	-25.03	-24.78	-24.97	-24.37	-25.02	-24.84	-25.14	-25.12	-24.92	-25.07	-25.68	-24.80	-24.31	-25.40
	-25.01	-24.74	-25.00	-24.41	-25.03	-24.85	-25.07	-25.10	-24.93	-25.02	-25.58	-24.81	-24.29	-25.48
C4 sugars	3.02	1.96	0.00	0.00	0.00	0.00	0.00	2.54	0.00	4.58	0.00	1.44	1.52	4.46
HMF (mg/kg)	6.46	13.31	12.55	4.29	3.84	5.44	7.68	2.18	5.38	8.52	42.82	3.14	4.93	8.83
	6.40	13.51	12.61	4.29	3.71	4.93	8.00	2.05	5.19	8.52	43.07	3.01	5.19	8.77
Proline (mg/kg)	804.56	815.07	795.22	689.45	666.67	645.63	949.46	955.89	812.15	972.83	647.97	479.70	647.39	478.53
	802.22	814.49	798.13	692.96	666.67	643.88	954.13	959.98	811.57	972.83	644.46	480.28	649.72	481.16
Acidity (meg/kg)	27.27	17.55	16.68	26.88	29.09	17.12	27.09	31.60	29.76	26.92	31.38	24.00	26.44	23.39
	27.88	17.61	16.68	27.08	29.70	17.05	26.91	31.20	29.86	26.97	31.26	23.85	26.40	23.49
Diastasis number	16.49	20.00	19.08	15.87	15.74	12.37	21.23	19.14	17.75	15.15	12.88	16.82	15.31	17.68
	16.00	19.91	19.65	16.01	15.16	12.38	21.34	16.53	17.03	15.31	12.85	16.62	15.16	17.81
Moisture (%)	15.1	13.6	14.1	17.2	16.1	14.1	16.1	15.7	16.9	14.0	14.7	15.5	16.7	16.5
Commercial glucose	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Naphthalene (mcg/kg) $^{(+++)}$	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Chloramphenicol (mcg/kg) $^{(++++)}$	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Fructose (g/100g)	38.59	42.65	40.86	39.90	38.90	39.87	39.59	38.62	38.78	38.37	42.15	41.08	39.72	36.61
	38.72	42.68	40.75	40.00	39.03	39.93	39.60	38.57	38.83	38.41	42.24	41.25	39.70	36.76
Glucose (g/100g)	30.42	30.54	30.90	32.71	33.19	34.76	32.59	31.16	35.59	35.35	38.83	33.65	31.74	31.22
	30.38	30.66	31.01	32.74	32.94	34.72	32.14	31.11	35.53	35.36	38.83	33.67	31.77	31.12
Sucrose (g/100g)	0.08	0.24	0.05	0.02	0.03	0.52	0.05	0.04	0.11	0.03	0.06	0.02	Nd	0.21
	0.07	0.25	0.06	0.02	0.03	0.50	0.06	0.04	0.10	0.02	0.05	0.02		0.18
Maltose (g/100g)	2.07	2.26	2.19	1.47	1.54	1.92	1.73	1.86	0.99	1.07	1.64	1.74	1.69	2.08
	2.04	2.30	2.11	1.42	1.63	1.87	1.80	1.77	0.98	1.01	1.64	1.77	1.68	2.03
Conductivity (ms/cm)	0.47	0.25	0.20	0.34	0.38	0.37	0.39	0.56	0.41	0.40	0.52	0.30	0.51	0.46
	0.47	0.25	0.20	0.34	0.38	0.37	0.39	0.56	0.41	0.40	0.52	0.30	0.52	0.46

Nd: not determined, $^{(++)}$ ^{13}C analysis were performed by national weather institute, $^{(+++)}$ minimal determination limit of naphthalene is 0.1 mcg/kg, $^{(++++)}$ minimal determination limit of Chloramphenicol is 0.23 mcg/kg.

According to the result of the pollen analyses carried out on honey samples, these pollen grains were determined as dominant: Fabaceae in 25 samples, Rosacea in 4 samples, *Cynoglossum* in 3 samples, *Rhododendron* in 3 samples, and *Xanthium*, *Cistus*, *Hedysarum*, Ranunculaceae, Rhamnaceae, and Scrophulariaceae pollens in one sample. The taxa whose pollens exist in secondary amount are Fabaceae in 13 samples, Castanea in 6 samples, Rosacea in 4 samples, Salix in 4 samples, Rhamnaceae in 3 samples, Apiaceae, Brassicaceae, *Cistus*, Lamiaceae, *Plantago*, and Scrophulariaceae in 2 samples and *Xanthium*, Boraginaceae, Echium, *Hedysarum*, Poaceae, Rumex, Ranunculaceae, and Chenopodiaceae/Amaranthaceae in one sample.

According to the analyses results, Fabaceae was identified as the primary source of nectar and pollen for the honey of the territory. While Rosacea family has been determined as the second taxon, Apiaceae, Asteraceae, Boraginaceae, Brassicaceae, and Lamiaceae are the other taxa that are the sources of honey in territory.

Results of diastase, HMF, conductivity, proline, commercial glucose, acidity, moisture, purity, sugar components analyses and chemical properties of 14 honey samples to represent all honeys are presented in Table 4.

The honey exporting countries in Europe attach much importance to the pollen content of honey since the percentage of pollens having various minerals, vitamins and enzymes shows the quality of honey (Dalgıç 1994). Most of the honey produced in Turkey is multifloral. It is of importance to know about the quality and source of the honey that beekeepers separately harvest honey which they collect from different plants and regions (Erdoğan 2007). It is easy to market the honey whose source and quality is well known. Since there is no legal arrangement in Turkey related to this issue, beekeepers do not pay attention to this matter. Therefore, it is necessary to take measures encouraging beekeepers to make the harvest after each nectar flow (Erdoğan 2007). Besides, in order to create an awareness in the public and to inform consumers, the percentage of the pollen and other nutritive elements should be written on the jars. Due to the fact that some pollens cause allergic reactions in some people, it is of much importance for consumer health to know the pollen ingredient of honey and also to identify allergic pollens, if any. The honey of Eskişehir territory has been determined as multifloral in general. Knowing the taxa to which pollens belong may contribute to both to increasing the production amount of honey and removing the unwanted plants from the honey. It is recommended that hives are located near the nectary plants or the plants that give honey its distinctive character are grown near hives. There are some problems in honey production and export in Turkey that is an associate member of the European Union. The beekeeping activities in Turkey should be arranged through legislative regulations, education and training of beekeepers, incentives that will encourage beekeeping. Otherwise, the potential of beekeeping which is national wealth for the economy in Turkey will be negatively affected.

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