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Research Paper / Araştırma Makalesi

Bacteriological, Physicochemical, and Melissopalynologic Properties of Some Turkish Honeys

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ABSTRACT

The bacteriological, physicochemical, and melissopalynological properties of some Turkish honey samples obtained from beekeepers and markets were investigated in this study. *Shigella* spp., *Salmonella* spp., *Clostridium* spp., *Paenibacillus larvae*, *Bacillus* spp., total mesophilic and coliform bacteria were screened to determine bacterial populations in honeys. Total coliform bacteria, *Shigella* spp., and *Salmonella* spp. were not found. Furthermore, *Clostridia*, *Bacillus* and *Paenibacillus* were in low levels in most of the honey samples. For all samples, the contents of hydroxymethylfurfural (HMF), electrical conductivity, total acidity, ash, moisture, brix, total protein and invert sugar were varied from 0.71 to 175.18 mg/kg, 0.19 to 1.69 mS/cm, 23.00 to 46.46 meq/kg, 0.03% to 0.89%, 13.1% to 19.4%, 80.78% to 85.08%, 0.13% to 0.18%, 54.55% to 71.52%, respectively. As a result of the melissopalynological analyses, 52 different pollen species were found. Pollen taxa found in large numbers of honeys were as follows; *Castanea sativa*, *Centaurea*, Asteraceae, Brassicaceae, Ericaceae and Fabaceae. According to the results, honey samples tested in this study were good in bacteriological quality. But, we proposed that collaboration of producers and microbiologists is needed to further improve bacteriological quality.

Keywords: Bacteriological analysis, Melissopalynological analysis, Physicochemical analysis, Turkish honeys

Bazı Türk Ballarının Bakteriyolojik, Fizikokimyasal ve Melissopalinolojik Analizi

ÖZ

Bu çalışmada arıcılardan ve pazarlardan alınan bazı Türk ballarının bakteriyolojik, fizikokimyasal ve melissopalinolojik özellikleri araştırılmıştır. Ballarda bakteri popülasyonunu belirlemek için *Shigella* spp., *Salmonella* spp., *Clostridium* spp., *Paenibacillus larvae*, *Bacillus* spp., toplam mezofilik ve koliform bakterileri taranmıştır. Test edilen örneklerin tümünde toplam koliform bakteri, *Shigella* spp. ve *Salmonella* spp. bulunamamıştır. Ayrıca, örneklerin çoğunda *Clostridia*, *Bacillus* ve *Paenibacillus* düşük seviyelerde bulunmuştur. Tüm örneklerin hidroksimetilfurfural (HMF), elektriksel iletkenlik, toplam asitlik, kül, nem, brix, toplam protein ve invert şeker içeriği sırasıyla; 0.71-175.18 mg/kg, 0.19-1.69 mS/cm, 23.00-46.46 meq/kg, %0.03-%0.89, %13.1-%19.4, %80.78-%85.08, %0.13-%0.18 ve %54.55-%71.52 aralığındadır. Melissopallinolojik analizler sonucunda 52 farklı polen türü bulunmuştur. Ballarda çok sayıda bulunan polen taksonları; *Castanea sativa*, *Centaurea*, Asteraceae, Brassicaceae, Ericaceae ve Fabaceae. Sonuçlara göre, çalışmada test edilen bal örneklerinin bakteriyolojik kalitesi iyidir. Ancak, mikrobiyolojik kaliteyi daha da iyileştirmek için üreticilerin ve mikrobiyologların işbirliği gereklidir.

Anahtar Kelimeler: Bakteriyolojik analiz, Melissopalinolojik analiz, Fizikokimyasal analiz, Türk balları

INTRODUCTION

Honey is an important research topic because of the numerous health benefits and biological properties. Its biological importance is often associated with properties such as high osmotic pressure, low water activity, hydrogen peroxide; lysozyme, high sugar content, and high acidity. Because of these properties, the microorganisms cannot survive in honey. Although honey possesses unsuitable environment for microbial growth, the microbial contamination in honey is known [1-3]. While the primer sources of contamination are dust, air, pollen, soil and nectar, the seconder sources are human, insects, equipments, containers, wind, dust and water. Honey is also an important food and energy sources due to its rich content. For example, it contains fructose, glucose, sucrose, minerals and proteins. The chemical and physical properties of honey are related to its quality. The contents of protein, moisture, the values of hydroxymethylfurfural (HMF), pH, diastase, electrical conductivity, dioxin analysis, trace element levels of honeys and menaquinones (vitamin K2 homologues) are known [4-7]. Phenolic and flavonoid content have described in Turkish honeys from different botanical and geographical origins [8, 9]. The environmental factors such as vegetation or geographic situation change the properties of honey. Also, these properties can vary according to the type of honey. So, pollen analysis is important with regard to give information about the plant source of honey [9-11]. In addition to, the physicochemical features of honey are known very well [2, 12, 13]. Nevertheless, there is a little paper about microbial contamination in honey and most of these studies are focused on Clostridium spp. [2, 5, 14]. Honey analyses are done to prove quality, botanical and geographical origins of honeys. For these purposes, the melissopalinology, biological and physicochemical analyses are the most common methods. In our study, we were detected the properties of physicochemical and melissopalynological and the bacterial contaminations in honeys obtained from beekeepers and markets in Turkey. According to the literature, bacteriological analyses of Turkish honeys have not been investigated in detail. Main purpose of this present study was to reveal the bacteriological profile in our samples. We detected *Shigella* spp., *Salmonella* spp., *Bacillus* spp., Paenibacillus larvae and *Clostridium* spp. Diastase activity, acidity, electrical conductivity, moisture, brix, ash content, total protein and invert sugar of all honey samples was determined. The sugar composition of two honey samples was analysed by HPLC. Also, melissopalynological properties were investigated to obtain information about the plant sources of honey samples.

MATERIALS and METHODS

Honey Samples

We analysed twenty two honey samples in our study. They were purchased from beekeepers (D-coded) and markets (T-coded) in 2012-2013 (Table 1). The names of companies (T-coded) were not given.

Bacteriological Analysis

Plate count agar (PCA, Merck) and violet red bile agar (VRB, Merck) were used to detect aerobic mesophilic and total coliform bacteria, respectively. Mesophilic bacteria were incubated at 30±2°C and total coliforms were also grown at 35±2°C [5]. *Shigella* spp., *Salmonella* spp. and *Bacillus* spp. isolation were performed by Iurlina and Fritz [5]. *Paenibacillus larvae* was also isolated in honeys [15]. For isolation of spores of *Clostridium* spp., 20 g of honey was diluted with 100 mL sterile distilled water. After it was centrifuged at 7168-11200x*g* for 30 min in 20°C, the sediment was mixed in about 2 mL sterile water. Suspension was heated at 80°C for 15 min and was spread onto Sulfite polymyxcin sulfadiazin (SPS, Difco) agar. Plates were incubated under anaerobic conditions at 30±2°C for 7-10 days and black colonies recorded as these microorganisms [16]. Microbial counts were recorded as colony-forming units per gram of honey (cfu/g) in all manipulations.

Physicochemical Analysis

Diastase activity (Schade method), acidity, and electrical conductivity were detected by International honey commission method and HMF was also measured by UV-spectrophotometer (284-336 nm) [17]. The contents of moisture and brix were measured using Mettler Toledo RM40 refractometer [13]. Ash content (%) was calculated according to the described by Bogdanov [1] and Anonymous [18]. Total protein was determined by the method of modified Lowry [19]. Determination of invert sugar was performed by TS 3036 [20]. The value of pH was measured with WTW Inolab pH meter. The sugar composition of the samples was detected via HPLC. The samples were randomly selected. HPLC analysis of honeys was carried out at the TÜBİTAK Marmara Research Center Food Institute Instrumental Analysis Lab.

Melissopalynological Analysis

Melissopalynological analysis, plant origin and pollen content of honeys were determined as follows: After 10 g honey samples were mixed with 20 mL distilled water. The tubes were covered with parafilm and heated at 40-45°C for 10-15 min. The samples were centrifugated at 3388-4032x*g* for 10 min. The supernatant was carefully decanted, and then the sediment was treated with glycerin-gelatin mixture. Finally, this preparation on slides was examined under microscope [21, 22]. We followed up various palynological sources in the diagnosis of pollen of honey samples [23, 24]. Especially, 300 pollen reference preparations belonging to the most visited plants by honey bees were used in our study.

Table 1. The sources of honey purchased from beekeepers

Sample	Source	Type	Year
D1	Mesudiye/DATÇA	Flower honey	2012
D2	Mesudiye/DATÇA	Flower honey	2012
D6	Çelikhan/MALATYA	Flower honey	2012
D7	Marmaris/MUĞLA	Flower honey	2012
D10	Babadağ/DENIZLI	Flower honey	2012
D11	Burhaniye/AYDIN	Flower honey	2012
D12	Kuyucak/AYDIN	Flower honey	2012
D16	Çelikhan/MALATYA	Flower honey	2013
D17	Datça/MUĞLA	Flower honey	2013
D19	KASTAMONU	Flower honey	2013
D20	BARTIN	Flower honey	2013
D22	SİVAS	Flower honey	2013

RESULTS and DISCUSSION

Bacterial Detection

Honey, which is a bee product, has both health and economic value. While the economic value of honey is related to its chemical content, the importance of health is due to its microbiological content. As known, the factors such as the concentrated sugar, acidity, pH and other antimicrobial characters of honey inhibited microorganisms. But, some microorganisms resistant under conditions and survive in honey. Especially, if honey is not properly packaged, it will absorb moisture from the environment and will be perfectly suitable for supporting microbial growth. The results are presented in Table 2. According to the bacteriological analysis results, total coliforms were negative in all samples. In our all samples, *Salmonella* spp. and *Shigella* spp. were absent, too. The presence of coliform bacteria is considered an indication of pollution in foods and water. This result indicated that all honey samples were good quality. These results are in good agreement with the observations of Malika et al. [25] and Gomes et al. [26]. Presence of these bacteria was detected by some researchers [5, 26, 27]. For instance, Iurlina and Fritz [5] reported the coliform contamination in one sample. Total mesophilic bacteria were commonly used as a reference shelf-life parameter for food products. On the other hand, it has been claimed that the contamination of total mesophilic aerobic bacteria may have occurred during harvesting and extraction of honey [3]. Total mesophilic bacteria were detected in all tested samples (Table 2). Therefore, the hygiene conditions must be controlled during harvesting. In contrast of our results, Iurlina and Fritz [5] reported that the contamination for aerobic mesophiles (average 244 cfu/g) counts were high. In foods, presence of spore forming bacteria such as *Bacillus* spp, *Clostridium* spp. and sulfite-reducing Clostridia spp. is another indicatore for contamination from soil or air [2, 27]. Actually the spores of *Clostridia* spp. and *Bacillus* spp. may be found at low levels in honeys. Kokuba et al. detected *B. coagulans*, *B. megaterium*, *B. alvei* and *C. perfringens* in honeys [28]. Shakoori et al. found *B. subtilis* in all samples; *B. circulans, B. brevis*, *B. coagulans* in three samples and *B. alvei* in two samples [29]. Moreover, *B. cereus* and *Enterococcus faecium* were reported by Lopez and Alippi, and Ibarguren et al., respectively [30, 31]. Gomes et al. screened microbiological properties of commercial honeys from Portugal and found low microbial contamination [26]. It was reported that Argentina honey samples were contaminated with sulphite-reducing *Clostridia* spp. [2]. The presence of high levels of these bacteria in honey is serious health problem for human especially children. Especially, it is not desirable to have coliform spores in honey for infants less than one year of age. In our present study, while the spore contamination of *Clostridia* spp., *Bacillus* spp. and *Paenibacillus* spp. were found low levels in most of samples, spores of these bacteria weren't detected in some samples (D6, D11 and D16). *Paenibacillus larvae* species is an important disease agent that effect on honeybees [32]. The spores of *P. larvae* were detected in all samples except five honeys (D10, D11, D12, T2 and T7).

The contamination of *P. larvae* was recorded in honey by some researchers [28, 32, 33]. This data was shown that the sanitary of bee colonies must be controlled carefully by honeymakers. In brief, we considered that tested honeys in this study were good in bacteriological quality. Our findings confirmed earlier findings of Tornuk et al. [3]. In other words, the level of contamination in tested Turkish honeys was limited levels. But, hygiene conditions during harvesting were not at the desired level. We considered that the improvement of microbiological quality will happen with the cooperation of producers and microbiologists.

pH

Honey pH influences the stability and shelf-life and the low pH also inhibits microbial growth [35]. For this reason, the pH of honey is important physical properties. The pH values of honeys were ranged from 3.14 to 4.78 (Table 3). In general, honeys obtained from markets were in more acidic properties than honeys obtained from beekeepers. These pH values were in parallel with the findings of Tornuk et al. [3], Silva et al. [34] and Kayacier and Karaman [35].

Table 2. The results of bacteriological analysis of honeys (cfu/g)

	Honeys	Total mesofilic	*Bacillus* sp.	*P. larvae*	*Clostridium* sp.	Coliform bacteria	*Salmonella* sp.	*Shigella* sp.
BEEKEPERS	D1	<10	<10	<10	<5	Negative	Negative	Negative
	D2	<10	<10	<10	<5	Negative	Negative	Negative
	D6	<10	<10	<10	Negative	Negative	Negative	Negative
	D7	<10	<10	<10	0.75×10^1	Negative	Negative	Negative
	D10	<10	Negative	Negative	0.50×10^1	Negative	Negative	Negative
	D11	<10	Negative	Negative	Negative	Negative	Negative	Negative
	D12	<10	Negative	Negative	<5	Negative	Negative	Negative
	D16	<10	<10	<10	Negative	Negative	Negative	Negative
	D17	<10	<10	<10	<5	Negative	Negative	Negative
	D19	<10	<10	<10	<5	Negative	Negative	Negative
	D20	<10	<10	<10	<5	Negative	Negative	Negative
	D22	<10	<10	<10	<5	Negative	Negative	Negative
MARKETS	T2	<10	Negative	Negative	<5	Negative	Negative	Negative
	T3	<10	<10	<10	<5	Negative	Negative	Negative
	T4	<10	<10	<10	<5	Negative	Negative	Negative
	T7	<10	Negative	Negative	<5	Negative	Negative	Negative
	T8	<10	<10	<10	<5	Negative	Negative	Negative
	T14	<10	<10	<10	<5	Negative	Negative	Negative
	T15	<10	<10	<10	<5	Negative	Negative	Negative
	T16	<10	<10	<10	<5	Negative	Negative	Negative
	T17	<10	<10	<10	<5	Negative	Negative	Negative
	T18	<10	<10	<10	<5	Negative	Negative	Negative

Hydroxymethylfurfural (HMF) Contents

(HMF is derived from dehydration of certain sugars and it is practically absent in fresh food. But the levels of HMF in sugar-containing foods are influenced by several factors such as heat-treatments like drying or cooking, time of heating, pH, and floral sources. Therefore, it can be used as an indicator for freshness and excess heat-treatment [22, 36]. It can be found in low amounts in honey. According to TS 3036 [20] HMF value of honey should be no more than 40 mg/kg. But, the HMF content of some tested honeys in present study (D7, D10, D12, T2, T3, T4, and T7) was found to be higher more than 40 mg/kg (Table 3). We considered that these samples have been stored for a long time or exposed through heat processing. The range of HMF contents of other honeys analysed in our study was between 0.71 and 175.18 mg/kg. Yılmaz and Yavuz [37] reported a lower HMF range for Turkish honeys (0.0-20.4 mg/kg).

Electrical Conductivity, Ash Content and Acidity of Honeys

The electrical conductivity, ash content and acidity indicate the difference between honeys with different floral origins, mineral content and shel-life of honey [8, 38]. Feas et al. [39] have posited that the ash content gives a direct measure of inorganic residue after carbonisation, while electric conductivity measures all ionizable organic and inorganic substances. The electrical conductivity values ranged from 0.66 to 0.24 mS/cm for market honeys, while the electrical conductivity values for beekeepers honeys were between 1.69 and 0.19 mS/cm (Table 3). Codex Alimentarius declared that total acidity levels should be 50 meq/kg [38]. For market honeys, free acidity values ranged from 14.4 to 29.7 meq/kg; the lactone acidity was between 6.0 and 30.6 meq/kg while total acidity levels varied between 27.33 to 46.46 meq/kg. Total acidity, lactone and free acidity of honeys collected from beekeepers were ranged from 23.00 to 45.97, 3.00 to 26.60, and 17.00 to 29.00 meq/kg, respectively. As known, acidity increases the antioxidant activity of honey and decreases growth of microroganisms. However, several factors such as organic acids, floral and geographical origin cause difference in acidity values [40]. In our study, total acidity levels of honeys were within the allowed limits (50 meq kg−1). Moreover, our results were similar to those of Tornuk et al. [3]. Ash content of honeys is low and depends on floral type. In our study, ash content of selected honeys from markets and beekeepers varied from 0.06% to 0.30% and 0.03% to 0.89%, respectively.

Moisture and Brix Content

Moisture content determines quality and storage properties in honey processing industries. Therefore, it is an indicator of honey freshness, shelf-life and resistance against yeast fermentation [36]. The moisture values of all honey samples were ranged from 11.9 to 13.1% (Table 3). On the other hand, the moisture percentage of all samples was within the limits specified by the Codex Alimentarius [38]. In our study, the brix content of all samples was found as 79.09-86.02% (Table 3). These results were in agreement with previous reports [2, 35, 41].

Table 3. The results of physicochemical analysis of honeys

	HONEYS	HMF (mg/kg)	Moisture (%)	Ash amount (%)	Total protein (%)	pH	Acidity (meq/kg)				Electrical conductivity (mS/cm)	Brix Free acidity	Invert sugar (%)
							Free acidity	Lactone acidity	Total acidity				
BEEKEEPERS	D1	15.75±0.20	13.3±0.10	0.43±0.02	0.18±0.02	4.16±0.01	22.0±2.00	11.0±1.00	33.00±3.00		0.89±0.04	84.87±0.03	57.78±0.38
	D2	36.40±2.68	13.9±0.10	0.17±0.01	0.17±0.01	3.85±0.01	29.0±1.00	16.0±4.00	45.33±3.00		0.44±0.02	84.28±0.05	59.04±0.76
	D6	13.15±0.30	13.1±0.10	0.04±0.01	0.16±0.01	3.37±0.01	18.0±0.00	7.00±1.00	25.00±1.00		0.20±0.01	85.08±0.04	67.96±0.21
	D7	62.37±1.00	16.1±0.20	0.34±0.02	0.16±0.01	4.15±0.01	28.0±2.00	6.00±2.00	34.67±0.70		0.74±0.04	82.10±0.05	54.55±0.15
	D10	96.87±0.45	15.5±0.00	0.10±0.01	0.18±0.00	3.50±0.05	28.0±2.00	13.0±2.00	41.00±1.00		0.32±0.01	82.78±0.01	67.56±0.06
	D11	36.38±0.40	13.9±0.10	0.09±0.00	0.15±0.00	3.38±0.02	22.0±2.00	4.67±0.30	26.33±0.30		0.30±0.00	84.33±0.03	67.10±0.07
	D12	173.80±1.00	14.6±0.00	0.03±0.01	0.18±0.00	3.14±0.02	17.0±1.00	3.00±1.00	23.00±1.00		0.19±0.01	83.60±0.03	66.42±0.19
	D16	1.17±0.01	14.3±0.00	0.17±0.02	0.16±0.01	3.80±0.02	24.0±0.15	22.0±0.00	45.97±0.10		0.43±0.03	83.74±0.04	69.94±0.08
	D17	2.19±0.01	14.7±0.40	0.39±0.02	0.16±0.00	4.78±0.01	17.0±0.02	26.6±0.10	43.60±0.10		0.82±0.04	83.38±0.14	54.91±0.65
	D19	2.68±0.02	15.9±0.10	0.26±0.01	0.18±0.01	3.66±0.01	17.0±0.02	25.6±0.10	42.60±0.02		0.60±0.02	82.34±0.06	67.55±0.33
	D20	0.71±0.01	15.4±0.50	0.89±0.02	0.18±0.01	4.63±0.01	19.0±0.03	20.4±0.10	39.40±0.10		1.69±0.03	82.84±0.19	56.29±0.37
	D22	2.68±0.03	19.4±0.10	0.08±0.01	0.17±0.01	3.35±0.01	19.0±0.04	25.3±0.10	44.40±0.02		0.28±0.02	83.07±0.23	65.50±0.16
MARKETS	T2	175.18±1.00	15.8±0.10	0.13±0.01	0.16±0.01	3.52±0.05	17.9±0.50	14.9±3.70	32.80±1.40		0.36±0.01	82.44±0.10	63.02±0.23
	T3	85.93±1.91	15.2±0.20	0.09±0.01	0.16±0.01	3.38±0.04	25.0±2.00	11.0±2.00	36.00±0.00		0.30±0.01	83.05±0.02	63.42±1.60
	T4	66.78±1.02	14.6±0.00	0.18±0.01	0.16±0.00	3.76±0.05	19.0±2.00	9.9±1.30	28.93±0.27		0.45±0.01	83.57±0.05	59.67±0.39
	T7	88.84±0.05	17.7±0.10	0.17±0.01	0.13±0.00	3.40±0.02	29.7±0.50	10.0±1.00	39.33±0.67		0.44±0.02	80.57±0.07	65.06±0.17
	T8	19.23±0.05	15.1±0.10	0.30±0.01	0.14±0.00	3.84±0.05	21.0±1.00	6.0±0.00	27.33±0.67		0.66±0.02	83.27±0.06	59.70±0.45
	T14	10.88±0.01	16.2±0.20	0.14±0.01	0.17±0.01	3.86±0.01	14.4±0.30	30.6±0.60	44.92±0.52		0.38±0.02	82.68±0.44	65.02±0.74
	T15	29.35±0.00	16.2±0.30	0.16±0.07	0.16±0.01	3.52±0.01	20.9±0.14	25.0±0.50	45.90±0.46		0.41±0.01	82.05±0.11	71.52±1.09
	T16	32.12±0.03	16.8±0.00	0.14±0.01	0.16±0.00	3.78±0.02	16.9±0.20	25.7±0.30	42.61±0.01		0.39±0.01	81.51±0.03	65.41±0.36
	T17	19.70±0.01	17.5±0.20	0.06±0.01	0.16±0.00	3.96±0.02	16.4±0.03	27.5±0.10	43.93±0.04		0.24±0.02	80.78±0.21	66.34±0.51
	T18	13.13±0.01	16.5±0.10	0.25±0.01	0.17±0.00	3.80±0.01	21.1±0.10	25.4±0.40	46.46±0.05		0.58±0.02	82.39±0.45	56.67±0.68

Total Protein

Protein contents of all samples were determined as 0.13-0.19% (Table 3). In general, the results were within the range established by TS 3036 [20]. However, the present study results corroborated the report of Küçük et al. [13]. Honey contains about 0.2% protein and also possesses various enzymes such as a-amylase, invertase, catalase, glucose oxidase, and phosphatase, which is related to plant origin, pollen and nectar [41]. In other words, the protein content of honey indicates the floral origine.

Invert Sugar and Sugar Composition

Sugar composition depends mainly on the origin of nectar, geographical origin, processing, and storage. Sucrose can be hydrolyzed by acid or the enzyme invertase, yielding an equimolar mixture of glucose and fructose. This mixture is called invert sugar. The inverted sugar is sweeter than sucrose [41, 42]. The invert sugar contents of commercial and natural honeys also were varied from 53.59% to 71.52% and 49.70% to 69.94%, respectively (Table 3). This results were similar to the invert sugar values obtained by Yardibi and Gümüş [42] and Kahraman et al. [43]. Invert sugar value (fructose and glucose) of the honey depends on the origin of the nectar [44] and invert sugar should be a minimum of 60% according to TS 3036 for flower honey [20]. In present study, 9 of 18 (50%) of commercial samples and 10 of 22 (45.45%) of natural samples were above this limit. The sugar composition of D6, D14 and D16 samples was also quantified and characterized by HPLC. The composition of D6 and D16 was slightly same. Sucrose, fructose, glucose and maltose were detected. Samples were composed mainly of glucose and fructose (Table 4).

Melissopalynological Analysis

Honey is classified by the floral source of the nectar from which it is made. The melissopalynological analysis can be determined pollen type and quantity, honey quality, botanical and geographical origin of honey, whether or not a fake [44]. Hence, the pollen analysis in honey is very important for determining the primary floral source. In general, numerous pollen types were detected in tested honeys (Table 5). While the botanical families such as Apiaceae, Fabaceae, Asteraceae, Brassicaceae, *Centaurea*, Ericaceae, Lamiaceae, Rosaceae, Poaceae, and Cistaceae were identified in honeys from beekeepers, the botanical families of honeys from markets were Apiaceae, Fabaceae, Asteraceae, Brassicaceae, *Centaurea*, Ericaceae, Lamiaceae and Amaranthaceae. About 52 different pollen taxa were found in samples. The richest pollen diversity was detected in flower honey samples T7. Identified pollen species, *Centaurea*, Asteraceae, Brassicaceae, Lamiaceae and Fabaceae were detected as the seconder taxa in most of honey samples. Pollens of *Citrus*, Brassicaceae, *Centaurea, Helianthus, Cistus, Erica,* Ericaceae, Lamiaceae, *Papaver, Pinus, Plantago,* Apiaceae, Fabaceae, *Ranunculus,* Amaranthaceae, *Echium, Eucalyptus, Daucus carota, Capparis, Astragalus, Arbutus*, *Anthemis*, *Morus*, *Lonicera*, *Rubus canescens*, and *Pistacia* were determined in minor amounts in some examples. Pollen taxa found in large numbers in flower honeys were as follows; *Castanea sativa, Centaurea*, Asteraceae, Brassicaceae, Ericaceae and Fabaceae. In our previous study, Amaranthaceae, *Trifolium*, *Trigonella*, Cyperaceae, *Zea mays*, *Anthemis*, *Papaver*, *Rumex*, *Trigonella*, Onopordum, and Apiaceae were found in pine honeys, while floral honey samples were characterized by *Erica*, *Centaurea*, *Helianthus annus* and Apiaceae [10]. Maia is named as monofloral if honey contains pollen in quantities exceeding 45% on the remaining pollen identified [45]. Usually one or more secondary pollen types in relation to numerous minor pollens were identified in our samples except for some honeys. For example, the flower sample D19 and D20 contained one secondary pollen type (*Castanea sativa*), but it wasn't characterized by dominant pollen. Thus, it was defined as multifloral. On top of it, Gomes et al. [26] claimed that chestnut honey should contain 90% of *Castanea sativa* pollen. Ozkok et al. evaluated melissopalynological similarities of 28 monofloral honeys and they reported that botanical similarity of all honey samples was 62.6% [9].

Table 4. Sugar composition of honey samples (g/100 g)

Samples	Sucrose	Fructose	Glucose	Maltose
D6	0.16	41.99	31.91	1.64
D16	0.21	41.71	31.74	1.50

Table 5. The results of melissopalynologic analysis of honeys

	HONEYS	Secondary pollen (16-45%)	Important minor pollen (3-15%)	Minor pollen (<3%)
BEEKEEPERS	D1	*Cistus,* Fabaceae	*Centaurea,* Asteraceae, Brassicaceae, *Erica,* Lamiaceae, *Morus, Pistacia*	*Anthemis, Citrus,* Poaceae, *Helianthus,, Plantago,* Rosaceae, Apiaceae, *Verbascum, Vitex, Washingtonia*
	D2	*Cistus,* Asteraceae	*Centaurea,* Brassicaceae,, Lamiaceae, Fabaceae, *Pistacia*	*Anthemis,* Amaranthaceae, Cistaceae, *Citrus, Daucus carota, Erica,* Poaceae, *Helianthus,, Daucus carota, Ligustrum, Morus, Papaver, Pinus, Pistacia, Plantago, Ranunculus,* Rosaceae, *Rumex, Thymus,* Apiaceae,
	D6	Asteraceae, Brassicaceae, Fabaceae	*Centaurea,* Lamiaceae, Rosaceae	*Cirsium,* Cistaceae, *Daucus carota, Echium,* Poaceae, *Morus, Phlomis, Plantago, Thymus, Verbascum,*
	D7			*Anthemis, Capparis, Centaurea, Cistus, Citrus,* Asteraceae, Brassicaceae, *Daucus carota,* Ericaceae, *Genista,* Poaceae, *Helianthus,* Lamiaceae, Fabaceae, *Lonicera, Morus, Papaver, Plantago,* Rosaceae, *Rumex,*
	D10	*Centaurea,* Asteraceae, Fabaceae	Cistaceae, Brassicaceae, *Morus, Polygonum*	*Castanea sativa,* Amaranthaceae, *Cistus, Eucalyptus,* Poaceae, *Helianthus,* Lamiaceae, *Olea, Papaver, Pinus, Ranunculus,* Rosaceae, *Rumex, Thymus,* Apiaceae, *Verbascum, Vitex,*
	D11	Brassicaceae, Ericaceae	*Capparis, Centaurea,* Poaceae, Fabaceae, *Plantago,* Rosaceae, Apiaceae	*Helianthus, Papaver, Polygonum, Ranunculus, Vitex,*
	D12		*Centaurea,* Asteraceae, Fabaceae, *Papaver,* Rosaceae, *Vitex*	Poaceae, *Salix,*
	D16	Asteraceae, Fabaceae	*Centaurea,* Brassicaceae, Rosaceae,	*Anthemis, Cirsium,* Cistaceae, *Cistus, Daucus carota, Echium,* Poaceae, Lamiaceae, *Morus, Papaver, Plantago, Thymus, Verbascum,*
	D17	Asteraceae, Lamiaceae, Fabaceae	*Centaurea,* Brassicaceae, Apiaceae	*Anthemis, Capparis, Helianthus, Morus, Pinus, Plantago, Ranunculus,* Rosaceae, *Rubus canescens, Rumex,* Urticaceae, *Verbascum, Vitex, Washingtonia*
	D19	*Castanea sativa,* Lamiaceae	Cistaceae, Asteraceae, Brassicaceae, *Fagus orientalis,* Fabaceae,	*Anthemis, Arbutus, Centaurea,* Amaranthaceae, *Cirsium, Cistus, Cotoneaster, Daucus carota, Diospyros lotus, Echium,* Poaceae, *Helianthus, Lonicera, Papaver, Pinus, Plantago, Ranunculus, Rhododendron, Rosa canina, Rubus canescens, Thymus,* Urticaceae,
	D20	*Castanea sativa,* Asteraceae, Brassicaceae	*Centaurea*	*Arbutus, Cirsium,* Cistaceae, *Cistus, Daucus carota, Fagus orientalis, Rhododendron,*
	D22	*Astragalus,* Lamiaceae, Fabaceae	*Centaurea,* Asteraceae, *Rubus canescens*	*Achillea, Echium, Papaver, Plantago, Ranunculus,* Rosaceae, Apiaceae,
MARKETS	T2	Asteraceae, Fabaceae	*Centaurea,* Brassicaceae, *Helianthus, Lamiaceae*	*Capparis,* Amaranthaceae, *Cistus, Echium, Eucalyptus, Geranium,* Poaceae, Lamium, Papaver, Pistacia, Rosaceae, Rumex, Thymus, Apiaceae, Verbascum, Vitex
	T3	Centaurea, Asteraceae	Brassicaceae, Fabaceae, Papaver, Apiaceae	*Anthemis, Castanea sativa,* Amaranthaceae, *Cistus,Cyperaceae, Dipsacaceae, Echium, Poaceae, Helianthus, Jasminum,Lamiaceae, Morus, Polygonum, Ranunculus, Rosaceae, Rumex, Verbascum, Xanthium strumarium*
	T4	Centaurea, Asteraceae, Brassicaceae	Cistus, Erica, Lamiaceae, Fabaceae	Anthemis, Poaceae, Helianthus, Morus, Pistacia, Plantago, Rosaceae, Thymus, Apiaceae, , *Verbascum, Vitex, Xanthium strumarium*
	T7	Centaurea, Asteraceae, Brassicaceae	Cistus, Fabaceae	Anthemis, Capparis, Chenopodiaceae, Cyperaceae, Erica, Eucalyptus,Poaceae, Helianthus, Jasminum, Morus, Papaver, Pistacia, Plantago, Ranunculus, Rosaceae, Rumex, Apiaceae, Verbascum, Vitex, Xanthium Strumarium
	T8	Centaurea, Asteraceae, Brassicaceae, Fabaceae	Helianthus	Anthemis, *Castanea sativa,Citrus,* Echium, Ericaceae, Eucalyptus, Morus, Pinus, Thymus, Apiaceae, Vitex
	T14	Asteraceae, Ericaceae	Centaurea, Brassicaceae, Echium, Eucalyptus, Helianthus, Lamiaceae, Lonicera, Morus	Achillea, Capparis, Amaranthaceae, Cistaceae, Ligustrum, Pinus, Washingtonia
	T15	Centaurea, Fabaceae	Arbutus, Astragalus, Capparis, Brassicaceae, Daucus carota, Lamiaceae	Achillea, Amaranthaceae, Cirsium, Cistacea, Echium, Ericaceae, Morus, Pistacia, Ranunculus,Plantago, Rosaceae, Rubus canescens, Apiaceae, Vitex, Washingtonia
	T16	Astragalus, Centaurea	Capparis, Brassicaceae, Ericaceae	Achillea, Cistus, Daucus carota, Eucalyptus, Poaceae, Helianthus, Morus, Papaver, Pinus, Plantago, Rosa canina, Rubus canescens, Rumex, Apiaceae, Vitex
	T17	Centaurea, Fabaceae	Amaranthaceae, Brassicaceae, Ericaceae, Pistacia	Achillea, Anthemis, Capparis, Cistus, Daucus carota, Echium, Erica, Eucalyptus, Poaceae, Papaver
	T18	Astragalus, Centaurea,	Amaranthaceae, Asteraceae, Daucus carota, Ericaceae, Rubus canescens	Achillea, Arbutus, Castanea sativa, Cistus, Cotoneaster, Eucalyptus, Poaceae, Lonicera, Morus, Pinus, Ranunculus, Thymus, Urticaceae

CONCLUSION

The result of analysis was showed that honey samples tested in study were good in bacteriological quality except for a few examples. Undouptely, quality and content of honeys will be different from each other due to many factors such as geographical origin, floral source, bee type, seasons, processing conditions, and storage period of honey. For high quality honey production, the education of beekeepers is very important. The standardization can be achieved by providing continuous training to the producers by experts.

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