Progress in Nutrition 2019; Vol.21, N. 4: 928-934 DOI: 10.23751/pn.v21i4.8862

Quality evaluation and pollen profile of honey samples from different locations

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Summary. In this study, it is aimed to determine some physicochemical properties and pollen types of honey samples collected from different regions. Pollen profile, moisture content, HMF, proline, frutose/glucose and electrical conductivity were the parameters analysed in each honey sample. According to the results obtained, all honey samples obtained from different geographic origins were found to be consistent, in terms of the parameters analyzed, with the values given in the Turkish Food Codex Honey Communiqué (2012). It was found that only one honey sample did not comply with the values given in the codex in terms of frutose/glucose ratio. As a result of the melissopalynological experiment, three honey samples were found to be unifloral (two *Castanea sativa* and one *Astragalus* spp.) and the others were found to be polyfloral honey. The results obtained indicate that physicochemical properties of the honey samples produced at different points in Turkey differ greatly in accordance with the diversity of the flora of the region.

Keywords: Honey, melissopalynology, proline, HMF, electrical conductivity

Introduction

Beekeeping is one of the most widely carried out agricultural activities today due to the importance of the products obtained. Honey, pollen, propolis, royal jelly, bee bread, bee venom and apilarnil are among the products produced as a result of this activity. These products are used mainly by people through health-protective functional properties they own. Therefore, different researches have been conducted on the physicochemical properties and biological activities of different bee products (1-6). Honey is the natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature (7). Honey has an important place in traditional medicine for centuries. The chemical composition of honey is complex, containing approximately 181 substances including proteins, moisture, sugars, minerals, enzymes, 5-hydroxymethylfurfural (HMF), vitamins, flavonoids, phenolic acids and volatile compounds (8). Individual groups of honey (blends, blossom, and honeydew) vary significantly in aroma, colour and taste. There are also differences in the chemical composition which are reflected in many physicochemical properties, such as in the content of ash, electrical conductivity, the spectrum of sugars, the activity of enzymes and pollen types (9).

Chemical and physical properties of different types of honey have been reported by many scientists (10, 11). Criteria such as moisture, water-insoluble content, free acidity, proline, electrical conductivity, HMF, diastase number, fructose/glucose, fructose+glucose, and the amount of naphthalene are used to determine the quality of honey (12). These characteristics of honey vary according to their plant and geographical origin, but are also influenced by certain external conditions such as season, processing, packaging and storage (13). Therefore, the present work was conducted to characterize the quality of honey samples from Turkey (n = 12) in terms of plant sources and physicochemical properties.

Material and Methods

Collection of honey samples

Honey samples of honey bee (*Apis mellifera* L.) were obtained from apiaries at 12 different province of Turkey in 2018 for determination of quality parameters and pollen types. All samples were stored at room temperature until analysis.

Pollen analysis

The pollen analysis of honey samples was based on the method described by Louveaux et al (1970) (14). Pollen types were classified into three categories: dominant pollen (>45%), secondary pollen (16–45%), important minor pollen (3–15%) and minor polen (<3%). When one pollen type represented >45% of the total number of pollen grains, the sample was classified as a monofloral honey. Although this classification is a generalization, for example, for the classification of chestnut honey as a monofloral honey it must contain 70% to 90% of *Castanea* pollen (15).

Physicochemical analysis

Moisture, sugar, proline and hydroxymethylfurfural (HMF) analyzes were performed according to the IHC (2009) (16). The electrical conductivity measurement of honey samples was carried out by using the method developed by Sancho et al (1991) (17).

Results and Discussion

In this study, pollen analysis of honey samples collected from 12 different provinces were carried out and plant taxa which were the source of these honeys were identified (Table 1). As a result of microscopic analysis of honey samples, pollen belonging to different families were identified at different rates. Most of these taxa belong to Fabaceae family. This indicates that bees prefer plants of this family in the regions where honeys are obtained and use them as nectar sources. Honey samples obtained from Bursa, Zonguldak and Tunceli provinces are grouped as unifloral honey because they contain dominant pollen grains, and these honeys are defined as *Castanea sativa* honey for honeys from Bursa and Zonguldak and *Astragalus* spp. honey for Tunceli honey. As a result of melissopalinological analyzes, honey samples obtained from 9 other regions were grouped as polyfloral honey. Fabaceae, Asteraceae, *Castanea sativa*, Cistaceae, *Cistus* spp., Fabacae, *Onbrychis vicifolia*, *Trifolium repens* and *Trifolium* spp. were among the taxa represented by secondary pollen in different honey samples. The pollens of *Brassica* spp., Apiaceae, *Astragalus* spp., Boraginaceae, Brassicaceae, Caryophyllaceae, *Castanea sativa*, *Centaurea* spp., *Cornus* spp, *Dianthus* spp., *Echium* spp., *Eryngium* spp., Fabaceae, *Hedysarum* spp., Lamiaceae, *Medicago* spp., *Melilotus alba*, *Melilotus* spp., *Minuartia* spp, *Nepeta* spp., *Rosa canina*, *Rosa* spp., Rosacaee, *Salix* spp., *Teucrium* spp., *Trifolium* spp. and *Verbascum* spp. taxa were represented in the honey samples in minor rate. In addition, pollens of 56 taxa were detected in trace amounts in honey samples. Similar to our study, melissopalinological examinations were conducted using honey samples from different locations of Turkey (18-21). Mercan (2007) (22) in their study, the pollen types of honey samples produced in the Aegean Region and around have determined. Pollen from Chenepodiaceae, *Trifolium* spp., *Trigonella* spp., Cyperaceae, *Zea mays* and *Anthemis* spp. were the most common ones. Dalgıç (1994) (23) examined 50 honey samples collected from different provinces of the Aegean Region between 1991 and 1993 in terms of biochemical and palynological. It was determined that the most common taxa of pollen in honey samples of this region were Fabaceae, Lamiaceae, Apiaceae, Brassicaceae families and *Helianthus annuus* from Asteraceae family, *Cistus* spp. from Cistaceae family and *Castanea sativa* from Fagaceae family. Bağcı and Tunç (2006) (24), as a result of pollen analysis of 21 honey samples collected from Konya region, Apiaceae, Rosaceae, Fabaceae (*Astragalus* spp., *Trifolium* spp., *Lotus* spp., *Onobrychis* spp.), Asteraceae (*Carduus* spp., *Centaurea* spp., *Achillea* spp., *Tragopogon* spp.), Brassicaceae (*Brassica* spp.), Lamiaceae (*Mentha* spp., *Salvia* spp.), Plantaginaceae (*Plantago* spp.) and Scrophulariaceae (*Linaria* spp.) determined that the important honey plants. Kaplan (1993) (25) deteced the pollen of Fabaceae, Brassicaceae, Rubiaceae, Euphorbiaceae family, *Salix* from Salixaceae, *Ranunculus* spp. from Ranunculaceae and *Centaurea triumfetti* from Asteraceae as the dominant in 24 honey samples collected from Konya region. Similar to this study, we identified pollen from different plant species in addition to the pollens belonging to

Onobrychis spp., *Astragalus* spp., *Salix* spp., Fabaceae and *Trifolium* spp. taxa in honey samples from Konya region. Göçmen and Gökçeoğlu (1992) (26) stated that the plants that contain the most nectar in the Bursa region are *Castanea sativa* from Fagaceae, *Helianthus annuus* from Asteraceae, *Daucus carota* from Apiaceae, *Rosa* spp. from Rosaceae, *Trifolium* spp. from Fabaceae and *Tilia argentea* from Tiliaceae. Similarly, in this study, we found that *Castanea sativa* pollen was dominant in the honey sample of Bursa origin and pollen belonging to *Cistus* spp., Fabaeae and *Rosa* spp. taxa were detected in minor rate. In a different research, Apiaceae, *Eryngium* spp. and *Scandix* spp. taxa pollen were found in important minor and minor amounts in 18 of 29 honey samples belonging to Erzincan region (27). It can be stated that the similarities and differences between our study and other studies stem from the floristic diversity of the regions where honey samples were obtained. The fact that bees visit different plant species rather than a single one as the source of nectar while creating honey indicates that this affects the taste, aroma, color and physicochemical properties of the honey. In addition to this, the type and proportion of secondary, important minor and even minor amount plant pollens explain that each honey has

Table 1. The pollen spectrum of honey samples

Location	Dominant	Secondary	Important Minor	Minor
İstanbul	-	Fabaceae Asteraceae	*Castanea sativa*	*Achillea millefolium* Apiacae *Anchusa* spp. *Brassica* spp. *Calluna* spp. *Centaurea* spp. *Cerinthe* spp. Dipsaceae Lamicaeae *Geranium* spp. *Malva* spp. *Onobrychis vicifolia* *Silene* spp. *Trifolium repens* *Trifolium* spp.
Sakarya	-	*Trifolium* spp.	Boraginaceae Brassicaceae *Castanea sativa* Lamiaceae *Melilotus* spp. Rosacaee	Apicaeae *Cistus* spp. *Eryngium* spp. Liliaceae *Mentha* spp. *Ornithogalum* Pinaceae *Ranunculus* spp. *Rosa* spp. Rubiacae *Salix* spp. *Tanacetum* spp. *Teucrium* spp. *Trifolium* spp.
Konya	-	*Onbrychis vicifolia*	*Verbascum* spp. *Hedysarum* spp.	*Astragalus* spp. *Centaurea* spp. Chenepodiaceae *Heliansthu*s spp. *Lotus* spp. *Medicago* spp. *Salix* spp. *Trifolium* spp.

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Table 1. The pollen spectrum of honey samples

Düzce	-	*Castanea sativa* *Trifolium repens*	*Brassica* spp. *Cornus* spp. Fabaceae *Melilotus alba* *Rosa canina*	*Acer* spp. Caryophylaaceae Lamiaceae *Pinus* spp. Rosacaeae *Salix alba* Scrophulariaceae
Bursa	*Catanea sativa*	-	-	*Cistus* spp. Fabacaee *Rosa* spp.
Trabzon	-	*Castanea sativa* *Cistus* spp. Fabaceae	Lamicaeae Rosaceae	Apicaeae Brassicaceae *Campanula* spp. *Hedysarum* spp. *Plantago* spp. Poaceae *Rubus* spp. *Rumex* spp. Scrophulariaceae
Zonguldak	*Catanea sativa*			Fabaceae Lamicaeae
Çanakkale	-	Fabacae	*Castanea sativa*	Apicaeae *Cistus* spp. *Olea* spp. *Papaver* spp. *Quercus* spp.
Balıkesir	-	Fabaceae	*Castanea sativa* *Echium* spp. *Trifolium* spp.	Apiaceae Asteraceae Boraginaceae *Cistus* spp. *Helianthus annuus* *Olea* spp. *Pinus* spp. Rosaceae *Stachys* spp. *Verbascum* spp.
Erzincan	-	*Astragalus* spp. Fabaceae *Onobrychis* spp.	*Centaurea* spp. *Dianthus* spp. *Eryngium* spp. Lamicaeae *Minuartia* spp. *Nepeta* spp. *Trifolium* spp. *Teucrium* spp. *Medicogo* spp.	Apiaceae Betula spp. Caryophyllace Liliaceace *Pinus* spp. *Quercus* spp. Rosacae Rosaceae *Taraxacum* spp.
Tunceli	*Astragalus* spp.		Caryophyllaceae Rosaeae *Salix* spp.	Apiacaee *Centaurea* spp. Liliaceae *Rumex* spp.
Şırnak	-	Fabacea Cistaceae	Apiaceae *Astragalus* spp. Lamiaceae *Rosa* spp.	Apiaceae Asteraceae Boraginaceae Caryophyllacace Cucurbiatace

different physicochemical properties. These differences might stem from the synergistic energy created by all these pollens that contribute to the formation of honey while contributing to honey.

The moisture content of the honey which is harvested after complete maturation is expected to be low. Water content of honey varies depending on harvest period, climatic factors and the degree of maturity reached in hive (28). As the moisture increases in honey, which is an important foodstuff, its quality decreases and the risk of fermentation increases. Therefore, ascertaining the moisture content of honey is one of the parameters used in determining the quality of honey. When the honey samples used in our study were examined, it was found that the moisture content in general varied between 15.3% and 18.9% (Table 2). Among the samples examined, honey samples with the lowest and highest moisture content were from Erzincan and Trabzon, respectively. The main sugars contained in honey are fructose and glucose, and in addition to these monosaccharides, it also contains disaccharides such as sucrose, maltose, isomaltose, lactose, and some oligosaccharides (29). The total amount/proportion of glucose and fructose in the content of honey is one of the parameters commonly used to detect adulteration in honey. In this study, the sugar ratios of honey samples were determined between 0.9 and 1.5, except one honey sample obtained from one region (Çanakkale) the sugar ratios of the samples were found to be compatible with the standard value (0.9-1.4) given in the Turkish Food Codex Honey Communiqué (2012) (12).

HMF amount is another important parameter for the evaluation of freshness and quality of honey, and generally not present in fresh honey, its content increases during conditioning and storage (30). In the Turkish Food Codex Honey Communiqué (2012) (12), it is stated that the HMF content of honey should be maximum 40 mg/kg in the blossom honey, and our results are consistent with this value. HMF contents of honey samples used in our study were found to be between 1.26-26.28 mg/kg and the highest rate was found in the honey sample obtained from Balıkesir region. Similarly, in their study conducted to determine the biochemical properties of highland honey and sunflower honey, Şahinler and Gül (2004) (31) determined the average HMF amounts of highland honey and sunflower honey as 5.73±0.18, 2.17±0.10 mg/kg, respectively and reported that all the samples were in accordance with the criteria specified in the Turkish Food Codex Honey Communiqué (2012) (12). Likewise, the amount of HMF was found in the range of 0.19-41.16 mg/kg in 49 different honey samples which were sold commercially in Southern Spain and were not heat treated, and it was stated that high amount of HMF resulted from the climatic conditions of Southern Spain (32).

In addition, proline values of honey samples were calculated in our study. According to this, proline values of honey samples were determined as 654 mg/kg (İstanbul), 712 mg/kg (Sakarya), 556 mg/kg (Konya), 949 mg/kg

Table 2. Quality characteristics of honey samples

	Location	Moisture (%)	HMF (mg/kg)	Proline (mg/kg)	F/G	Electrical conductivity (mS/cm)
1	İstanbul	18.3	6.44	654	0.9	0.49
2	Sakarya	17.6	3.52	712	1.1	0.47
3	Konya	16.5	11.34	556	1.3	0.26
4	Düzce	16.2	10.16	949	1.1	0.32
5	Bursa	16.0	4.80	740	1.3	0.92
6	Trabzon	18.9	8.86	652	1.0	0.11
7	Zonguldak	18.1	7.38	1055	1.2	0.98
8	Çanakkale	16.7	14.16	596	1.5	0.74
9	Balıkesir	17.3	26.28	728	1.3	0.70
10	Erzincan	15.3	4.99	628	1.0	0.25
11	Tunceli	16.2	18.24	856	1.4	0.56
12	ırnak	16.4	1.26	846	1.0	0.74

(Düzce), 740 mg/kg (Bursa), 652 mg/kg (Trabzon), 1055 mg/kg (Zonguldak), 596 mg/kg (Çankkale), 728 mg/kg (Balıkesir), 628 mg/kg (Erzincan), 856 mg/kg (Tunceli) and 846 mg/kg (Şırnak). The lowest proline value was determined to be 556 mg/kg in Konya honey and the highest value was determined to be 1055 mg/kg in chestnut honey (Zonguldak). Proline, which is regarded as one of the quality criteria of honey, is an amino acid that comes from honey bees and mixes with honey during the processing of nectar and shows the maturity of honey. Therefore, it is a parameter that reflects the botanical origin of honey (33, 21). The free amino acid concentration in honey is 100 mg/100g in average and 50-85% of the total amino acid amount is composed by proline (34). The results show that the proline contents of honey samples are significantly different depending on the regions. In addition to this, the electrical conductivity values of honey samples used in our study were determined between 0.11-0.98 mS/cm (Table 2). Electrical conductivity is an important parameter used to determine the source of the honey. In our study, the electrical conductivity value of honey samples was found to be consistent with the values of Turkish Food Codex Honey Communiqué (2012) (12) and it was determined between 0.92-0.98 mS/cm for chestnut honey and 0.11-0.74 mS/cm for other honey samples. Many studies investigating the quality characteristics of the honey produced in different regions of Turkey and the world have been conducted in parallel to our study (19, 35, 36). Similarly, in order to determine the quality of blossom honey samples, which are released to the market for consumption, it was determined that the average moisture content was 17.56%, the average fructose/glucose ratio was 1.22 and the average electrical conductivity value was 0.46 mS/cm in 50 honey samples (37). Muli et al. (2007) (38) examined the quality characteristics of 72 honey samples and the findings are as follows: the average moisture16.00%, HMF 3.70-389.36 mg/kg; proline 20.83-300.6 mg/kg. Ölmez (2009) (39) analyzed the moisture, glucose, fructose and HMF values of 8 different honey samples harvested in the same conditions from different regions of Turkey in 2006 and 2007 and the total of glucose and fructose was detected as 51.31-68.30%, HMF value as 1.34-31.28 mg/kg, moisture content as 17.1-20.0%. According to the results of our study, no honey sample has the same characteristics with another one, and therefore, it can be inferred that especially geographic differences affect the physicochemical properties of honey significantly. However, anthropogenic factors are also considered to have a significant effect on the physicochemical properties of honey.

Conclusion

It was observed that honey samples produced in different regions of Turkey, whose floral diversity was quite high, did not share the same quality. This can be explained by the differences in regions from which honey samples are collected and accordingly the diversity of plant species that make up the content of honey. Therefore, when the fact that the plant source has a significant effect on the physicochemical properties of honey is considered, making a plant source map of honey with regard to regions is very important in terms of revealing the differences of honey.

Conflict of interest

No potential conflict of interest relevant to this article was reported by the authors.

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