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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

Physicochemical Analysis Of Some Honey Samples From Konya And Karaman Regions

Konya ve Karaman İllerinden Bazı Bal Örneklerinin Fizikokimyasal Analizi

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ÖZET

Balın kimyasal yapısını ve kalitesini etkileyen birçok faktör vardır. Bu faktörlerden en önemlisi bala kaynak olan polen ve nektardır. Anadolu balları zengin bitki çeşitliliğinden kaynaklanan değişik polen tiplerine sahiptir. Balın kalitesini belirlenmesinde kullanılan yöntemlerden birisi de fizikokimyasal analizlerdir. Fizikokimyasal parametreler ise balın elektriksel iletkenliği, serbest asitliği, diastaz aktivitesi, nem içeriği, prolin miktarı, pH, fruktoz, glukoz, sukroz içeriği, polenin rengi ve tadıdır. Bu çalışma ile 2015 yılında Konya ve Karaman bölgesinin farklı lokalitelerinden toplanan 17 multifloral bal örneğinin kimyasal analizlerinin yapılarak kalitesinin belirlenmesi amaçlanmıştır. Analiz sonuçlarına göre, elektriksel iletkenlik 0.19-0.58mS/cm, serbest asitlik 18-29(meq/kg), diastaz sayısı 10.4-34.9, pH 3.63-4.72, nem içeriği %15.40-18.80, glukoz oranı %26.47-33.70, fruktoz oranı %35.51-40.19, fruktoz/glukoz oranı %1.10-1.41 ve prolin miktarı 349-908 mg/kg olarak belirlenmiştir. Tespit edilen sukroz ortalama değeri, standart değerlerle uyumlu görülmektedir. İncelenen tüm fizikokimyasal parametreler, Türk Gıda Kodeksi ve EU Kodeksi referans değerlerine göre normal değerlerde görülmektedir.

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ABSTRACT

There are many factors that affect quality and chemical composition of honey. The most important of these factors is pollen and nectar of blossoms or secretions of living parts of plants. Anatolian honey has various pollen types, because of rich plant diversity. Also physicochemical analysis is one of the procedure determining the quality of honey. These physicochemical parameters are electrical conductivity, free acidity, diastase activity, moisture, proline, pH, content of fructose, glucose, sucrose, colour of pollen and taste. The aim of this study was to determine the quality of 17 multifloral honey samples collected different locations from Konya and Karaman regions in the year 2015. The ranges of parameters in examined honey samples are 0.19-0.58mS/cm the electrical conductivity, 18-29 free acidity (meq/kg), 10.4-34.9 diastase activity, 3.63-4.72 pH, 15.40-18.80% moisture, 26.47-33.70% glucose, 35.51-40.19% fructose, 1.10-1.41 fructose/glucose and 349-908 mg/kg proline. The mean percentage of sucrose in honey samples is convenient with the standart value. The results of these parameters are in the normal ranges proposed by Turkish Food Codex (TFC), EU codex.

1. Introduction

Honey is a sweet natural product made by honeybees by using pollen and nectar of flowers or exudates from trees and with the addition of plant-sucking insects. Turkey has very favourable conditions for apiculture with its different climatic conditions, ecosystem diversity and more than 10 000 plant species. Therefore Anatolian honeys are rich in pollen types per sample and 85% of the world's floral types can be found in Turkish honeys (Gök et al., 2015). Karaman-Konya region where the working samples are collected are located within the Irano-Turanian pytogeographical region. This phytogeographic region has very rich in terms of plant species. Özbey et al., (2015) also identified macrophtic vegetation type, halopytic vegetation type, ruderal vegetation type and step vegetation type in the region. These different source of pollen and nectar affects the chemical composition of the honey in terms of its organic acid, enzyme, protein, carbohydrate content.

Physicochemical analysis are used to determine the quality of honey. For the quality criteria of honey, certain constituents such as moisture content, electrical conductivity, reducing sugars, amount of fructose and glucose, sucrose content, individual sugars, mineral, free acidity, diastase activity, HMF content, invertase activity, proline content and specific rotation have been proposed by the international honey commission (IHC) (Bogdanov et al.,1999; Joshi et al., 2000) . With increasing interest in honey composition, many studies have been carried out in relation to physicochemical parameters in the other countries (Anupama et al., 2003; Felsner et al., 2004; Dag et al., 2006; Finola et al., 2007; Qamer et al., 2008; Khalil et al., 2012; Aloisi, 2010; Shahnawaz et al., 2013; Akram et al., 2014; El-Shoimy et al., 2015). But there are limited studies on phsicochemical parameters although honey is widely consumed and used in ethnomedicine in Turkey (Yılmaz and Küfrevioğlu, 2001; Sorkun et al., 2002; Silici, 2004; Ünal and Küplülü, 2006; Küçük et al., 2007; Doğan, 2008; Günbey et al., 2010; Erez et al., 2015).

This study focused on the effect of plant diversity on the physicochemical characteristics of honey samples collected from Konya and Karaman regions in Turkey.

2. Material And Method

Honey Samples

17 multifloral honey samples were obtained from local producers in different localities of Konya and Karaman regions (Middle Anatolia) in November 2015 (Fig. 1). If the honey contains pollens of multiple taxa, it is called multifloral honey. All samples were collected in sterile glass bottles (labelled with numbers, collection locality and date) and stored at room temperature until chemical and physical analysis were done.

Figure 1. Konya (A) and Karaman (B) regions from Turkey

Physicochemical Analysis

In this survey free acitidy, proline, sugar profiles, moisture, pH, diastase activity and electrical conductivity were examined according to methods proposed by TS13360 (Anonymus, 2008), TS13359 (Anonymus, 2008), AOAC (AOAC 1990) and Bogdanov et al. (1997) respectively. Electrical conductivity was determined on a 20% w/v honey: water solution on a dry matter basis and expressed in mS/cm (Vanhanen et al., 2011). The conductivity measurements were conducted using a conductivity meter (Thermo Scientific, Orion 3 Star). Moisture was determined by measuring the refractive indices at 20o C using digital refractometer (Atago, RX 5000α) and the corresponding moisture contents (%) were calculated (Khalil et al., 2012).

The pH value was measured with a pH-meter (Sartorius) in solution of 10 g honey in 75 ml CO2-free distilled water (Shahnawaz et al., 2013). Free acidity was determined using the titrimetric method. Aqueous honey solution (10 g in 75 mL distilled water) was titrated with NaOH until pH 8.3, after adding

phenolphthalein (Khalil et al., 2012). Diastase activity was measured using Phadebas® tablet and spectrophotometer at 620 nm wave length (Anonymus, 2009). Sugar profile that was used in the analysis was determined by HPLC method according to TS 13359 (Anonymus, 2008).

Statistical Analysis

To classify examined honey samples based on ten pyhsicochemical variables, multivariate analyses of hierarchical cluster analysis was applied (HCA) (IBM Corp. Released SPSS Statistics 21.0, 2012). In HCA, the Euclidean distance with complete linkage rule was used to group honey samples in clusters in terms of their nearness with linkage distance or similarity (Fig. 2) (Kek et al., 2016).

3. Results and Discussion

In this study, physicochemical characteristics of 17 honey samples from Turkey were analysed and compared to the values set by Turkish Food Codex (TFC) (Turkish Food Codex 2012), EU codex (European Union, 2001). The obtained results from analysed honey samples were given in Table 1.

It was observed that the range of the moisture contents were various from 15.40 % to 18.80% which are within the limit (≤20%) recommended by TFC, EU codex (Table 1). The moisture content varies depending on specific composition of honey, the ratio of sugar content and amount of water. These results are in agreement with the findings of Küçük et al. (2007) who reported that the average moisture content was recorded 17.0% in heterofloral honey, 19.7% in Chestnut honey, 19.0% in Rhododendron honey. Günbey et al. (2010), Ng'ang'a et al. (2013) and Derebaşı et al. (2014) found that the range of moisture content in different honey samples was 16.12%, 16.87 and 19.13% respectively. Although Khalil et al. (2012) measured lower moisture content in Algerian honey samples (11.59 and 14.13%), Akram et al. (2014) measured higher moisture content in different honey samples of Apis dorsata in different locations of Pakistan (22.87-26.70%).

According to White (1979), honey contains about 200 substances. Honey is composed of approximately 80% sugar (mainly glucose and fructose), 17% water and organic acids, mineral salts, vitamins, proteins, phenolic compounds, lipids and free amino acids, pollen, beeswax and pigments. The mineral content and elements in trace amounts of honey can be used in determining the geographical origin.

Samples No	Proline (mg/kg)	Fructose %	Glucose %	Fructose/ Glucose	Sucrose %	Moisture %	pH	Electrical conductivity	Diastase number	Free Acidity
1	654	38.87	30.12	1.29	nd	17.60	3.79	0.324	29.3	24.00
2	653	39.97	30.72	1.30	nd	16.36	3.93	0.281	22.7	20.00
3	559	39.34	31.10	1.27	nd	17.87	3.70	0.196	15.6	18.00
4	707	40.19	30.89	1.30	nd	17.97	3.74	0.249	33.6	23.00
5	607	37.07	31.55	1.18	0.05	16.54	3.65	0.307	13.2	20.00
6	545	39.56	31.85	1.24	nd	17.41	3.63	0.251	22.7	22.00
7	568	39.46	33.70	1.17	0.14	18.09	3.67	0.338	21.0	23.00
8	349	38.42	27.22	1.41	nd	17.27	4.72	0.587	22.4	22.00
9	492	39.46	29.93	1.32	nd	16.51	4.38	0.463	23.6	22.00
10	908	40.12	31.50	1.27	nd	15.40	3.91	0.425	34.9	20.00
11	513	38.18	32.50	1.17	0.10	18.59	3.86	0.360	13.2	19.00
12	542	35.51	32.33	1.10	0.13	16.38	3.80	0.396	10.4	20.00
13	781	37.31	26.47	1.41	nd	15.74	4.24	0.539	23.2	29.00
14	830	39.07	27.93	1.40	0.04	17.33	3.75	0.274	24.4	21.00
15	552	38.01	30.11	1.26	nd	18.80	3.85	0.312	19.5	29.00
16	538	37.52	28.24	1.33	nd	18.38	4.16	0.325	14.2	19.00
17	463	38.96	33.03	1.18	nd	16.59	3.90	0.422	14.6	20.00
TFC, EU Codex Standart	>300[1] >300[2]	Not fixed limit	Not fixed limit	0.9-1.4[1] 1.0-1.4[2]	<5[1] >10[2]	<20[1] <20[2]		<0.8[1] >0.8[2]	>8[1] >8[2]	<50[1] <50[2]

1= Blossom honey, 2= Honeydew honey, nd=not dedected

Table 1. The results of physicochemical analysis of honey samples from Konya and Karaman regions of T

The carbohydrate content in honey is approximately 80%. Glucose and fructose in honey is about 75%. Maltose, melositose and oligasacharites are other carbohydrates in honey. While the amount of fructose is high in many honey types, the amount of glucose comes second. The amount of sucrose is dependent on the amount of invertase enzyme (Anonymus, 2016). The result of analysis showed that the sucrose ratios were in normal range.

The results of analysis of all the seventeen (17) honey samples indicated that the ratio of fructose in the examined honey samples ranged from 35.51 to 40.19%, and the ratio of glucose ranged from 26.47 to 33.70%. The ratio F/G ranged from 1.10% to 1.41%. As it is seen from the results, the amount of fructose was higher than the amount of glucose. These obtained results supported the previous studies (El Sohaimy et al., 2015; Buba et al., 2013). Based on this subject some authors have stated that when the glucose content was lower than the fructose content, honey colonies were feeding naturally (El Sohaimy et al., 2015; White and Doner, 1980). The sum of fructose and glucose ranged from 65.64 to 73.16%. While the value of sucrose in 12 samples was convienent standard limit, it was below the standard limit in 5 samples (Table 1). Derebaşı et al. (2014) explained that a high sucrose concentration of honey, most of the time, means an early harvest of honey because sucrose has not been fully transformed to glucose and fructose by the action of invertase. The sucrose value of different honey samples had been found as 3.91%, 5.24%, 1.47%, 0.49-9.77%, 4.12%, 2.29%, 0.35-16.29%, by Sorkun et al. (2002), Silici (2004), Küçük et al. (2007), Doğan (2008), Günbey et al. (2010), Khalil et al. (2012), Derebaşı et al. (2014) respectively.

In the present study, electrical conductivity (EC) of honey samples was between 0.2 and 0.6 mS/cm. This parameter varies depending on the resource of nectar and the amount of organic acid, mineral salts and protein content of honey. Bogdanov et al. (1999) declared that electrical conductivity is good feature to use in order to separate the flower honey from honeydew honey for the characterization of unifloral honey. This measurement depends on the ash and acid content of honey; the higher ash and acid content, the higher the resulting conductivity. Blossom honey, mixtures of blossom and honeydew honey should have less than 0.8 mS/cm while pure honeydew honey and chestnut honey should have more than 0.8 mS/cm. EC was found 0.53 -4.18 for honey from different origins, 1.67-10.90 for Trifolium honey from Turkey, 0.12–2.42 for from black sea region from Turkey, 0.05 mS/cm for Pakistani honey samples, and 117-428 mS/cm for Bulgarian honey (El Sohaimy et al., 2015; Doğan, 2008; Derebaşı et al., 2014; Rahman et al., 2010; Atanassova et al., 2009).

The pH of honey varies according to the presence of organic acids. Acidity contributes the taste of honey, stability against micro-organisms, to increase chemical reactions in honey, antibacterial and antioxidant activities (Anonymus, 2016). The pH values in the studied honey samples were acidic which varied between 3.63 and 4.72 (Table 1) and within Turkish honey standard (3.4-6.1) (Anonymus, 2010). Our results showed close resemblance to pH values measured as 3.70-4.00 by Khalil et al. (2012) and as 3.82-4.43 by Ng'ang'a et al. (2013). The pH values of analysed honey have been previously reported as 3.16-4.77 in Turkey (Doğan, 2008), 4.114-4.637 from Egypt, Yemen, Saudi Arabia and Kashmir (El Sohaimy et al., 2015), 4.17 in Argentina (Aloisi 2010) and 4.50-6.00 in Turkey (Derebaşı et al., 2014). Derebaşı et al. (2014) pointed out that the pH was indeed a useful index of possible microbial growth, since most bacteria grow in a neutral and mildly alkaline environment, while yeasts and moulds were capable of developing in an acidic environment (pH 4.0–4.5), and did not grow well in alkaline media. According to Bogdanov et al. (2004), all honeys are acidic with a pH-value generally lying between 3.5 and 5.5.

The acidity of honey is based on mainly gluconic acid and other organic acids. Gluconic acid is produced from enzymatic breakdown of glucose by glucose oxidase which is found naturally in honey (Akram et al., 2014). The variation of free acidity in honey can be diverse according to harvest season and regional flora (Erez et al., 2015; Derebaşı et al., 2014). Free acidity values of 17 honey samples studied range from 18.00 to 29.00 meq/kg (Table 1) and all honey samples analysed were below the limit proposed by TFC and EU (<50 meq acid/kg). Our results were closer to data of Sorkun et al. (2001) (24.34-32.81 meq/kg), Finola et al. (2007)(11.9-29.4 meq/kg), Derebaşı et al. (2014) (17.00-34.00 meq acid/kg) and Erez et al. (2015)(16.41-26.20 meq/kg). However, the value of free acidity (15.51-64.68 meq/kg) in Trifolium honey from Turkey declared by Doğan (2008) were higher than our results.

The source of protein and amino acids in honey is mainly pollens. Proline that is the main amino acid in honey, added to honey by the bee in different unifloral honeys show various characteristic values that is correlated with enzymatic activity (Bogdanov et al., 2004). The proline content is used as a criterion of honey ripeness and, in some cases, sugar adulteration (Anonymus, 2009). The amino acid profile of the honey can be used in determining the botanical origin of honey. The amount of proline in quality honey should be higher than 350 mg / kg and at least 66% of the total amino acids (Anonymus, 2016). The results of honey samples examined showed that the proline content was found in the range of 349-908 mg/kg. While the amount of proline was found the lowest in sample 8, it was the highest in sample 10. It seems that our results are consistent with the standard (Table 1). Some authors determined that amount of proline was 324-673, 290-580, 430-734, 596-12.0, 305-650, 298-1199, 264-636, 329-931 mg\kg in Greek unifloral honey of different

botanical origins (Thrasyvoulou and Manikis, 1995), 68.85-116.10 mg/100 gr in Eucalyptus camaldulensis honey (Sorkun et al. 2001), 1692–2712 mg/kg in Algerian honey (Khalil et al., 2012) and 220±5.8, 192±6.4, 234±5.9 in multifloral honey (Erez et al., 2015).

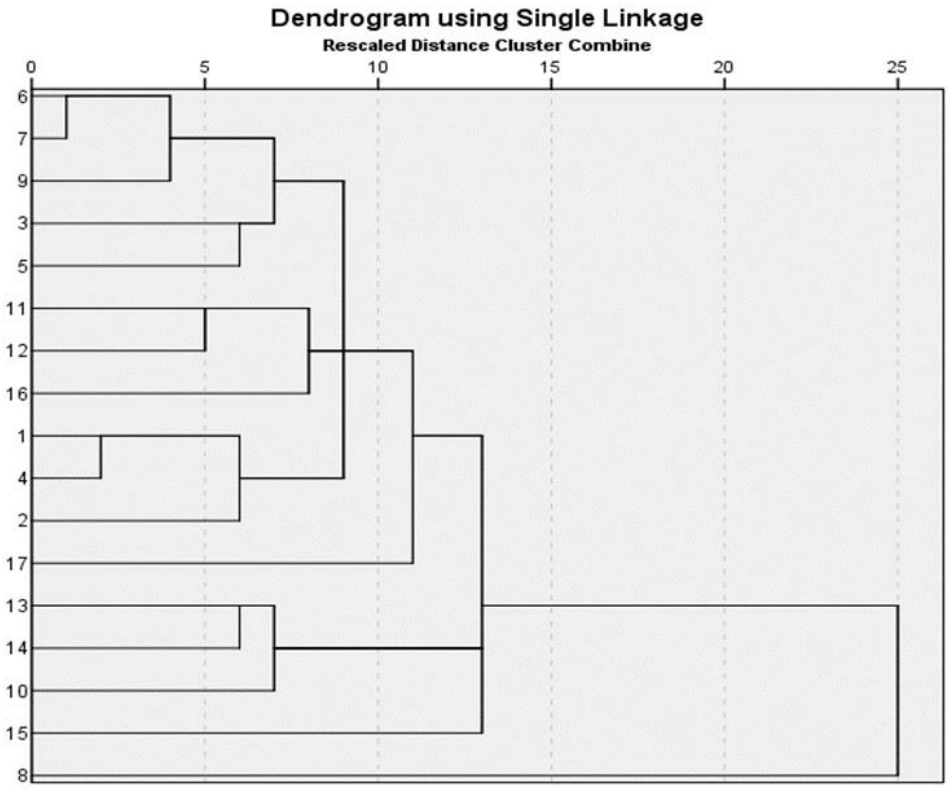

Figure 2. The dendrogram of cluster analysis for 17 honey samples based on physicochemical parameters

Enzymes are among the most important and interesting components that make up the content of the honey. There are small amounts of various enzymes in honey. Diastase is added to honey by bees and catalyses the transformation of starch to maltose. Honey diastase activity is a quality factor, influenced by honey storage and heating and thus an indicator of honey freshness and overheating (Bogdanov et al., 2000). The diastase activities in this study were ranging from 10.4 to 34.90 (Table 1). This value was significantly higher than the recommended quality criteria (>8) by FAO/WHO Codex, TFC and EU. Küçük et al. (2007) suggests that a high quality honey is expected to have high diastase activity. Higher diastase number was found in Christ's thorn honey (25.39-50.51DN) by Daniela et al. (2008); in Burkina Fasan honey (6.5-62.3DN) by Meda et al. (2005); in Trifolium honey from Turkey (13.90-50.00) by Doğan (2008); in Eucalyptus camaldulensis honey from Turkey (10.90-38.50) by Sorkun et al. (2001). Lower diastase number was detected as 22.68 by Sorkun et al. (2002), as 10.48 by Silici (2004) as 17.9 (heterofloral honey), 17.7 (chestnut honey) and 23.0 (Rhododendron honey) by Küçük et al.(2007) as 4.53-11.23 by Ng'ang'a et al. (2013) as 5.00-23.00 by Derebaşı et al. (2014).

The results of HCA showed that 17 honey samples could be classified big large groups. As can be seen in the dendrogram, subgroups in the large cluster have similar pyhsicochemical characteristics (Fig. 2).

The physicochemical analysis of honey samples obtained from Konya and Karaman regions indicated that honey samples had a good level of quality and all parameters were within the quality criteria according to TFC and EU Codex. We can conclude that the bees fed from natural plant resources and beekeepers in the regions carry out a conscious production.

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