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DETERMINATION OF TOTAL PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF ANZER HONEY PRODUCED IN RIZE, TURKEY

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ABSTRACT
The purposes of this study is to determine the physico-chemical and bioactive properties of Anzer honey, which is produced in widespread of Anzer plateau in Rize. Mineral contents of samples were determined by Inductivelly Coupled Plasma Optical Emission Spectrometer (ICP-OES). The most abundant minerals were potassium, calcium, sodium, and magnesium, ranging between 1265.87 - 5887.65, 299.56 - 854.36, 289.41 - 591.45, and 41.54 - 90.54 mg/kg, respectively. In addition, this study shows that the color of Anzer honey is quite different and it is usually light yellow and some of it is dark brown. The total phenolic content of the samples (TPC) was found between 802.6- 1352.6 µg GAE / g honey. Total antioxidant contents (TAC) were determined between 9.12-20.69 µmol TE / g. TAC of honey samples clearly shows that it contributes to TPC and sweeping activity.
Keywords: Anzer, ICP-OES, DPPH, TAC, TPC.

RİZE'DEKİ ANZER YAYLASI'NIN ENDEMİK ÇİÇEKLERİNDEN ÜRETİLEN ANZER BALININ TOPLAM FENOLİK BİLEŞİKLERİNİN VE ANTİOKSİDAN KAPASİTESİNİN BELİRLENMESİ

ÖZ
Bu çalışmanın amacı, Rize'deki Anzer yaylasında yaygın olarak üretilen Anzer balının fiziko-kimyasal ve biyoaktif özelliklerini belirlemektir. Numunelerin mineral içerikleri, İndüktif Eşleşmiş Plazma Optik Emisyon Spektrometresi (ICP-OES) ile belirlenmiştir. En fazla bulunan mineraller sırasıyla 1265.87 - 5887.65, 299.56 - 854.36, 289.41 - 591.45 ve 41.54 - 90.54 mg/kg arasında değişen potasyum, kalsiyum, sodyum ve magnezyumdur. Ayrıca, bu çalışma Anzer balı renginin oldukça farklı olduğunu ve genellikle açık sarı ve bazılarının koyu kahverengi olduğunu göstermektedir. Numunelerin toplam fenolik madde miktarı (TPC) 802.6- 1352. 6 µg GAE/g bal arasında bulunmuştur. Toplam antioksidan içerikleri de (TAC) 9.12-20.69 µmol TE/g arasında tespit edilmiştir. Bal örneklerinin yüksek antioksidan kapasitesi, fenolik bileşiklere (TPC) ve süpürme aktivitesine katkıda bulunduğunu açıkça göstermektedir.
Anahtar kelimeler: Anzer, ICP-OES, DPPH, TAC, TPC.

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INTRODUCTION

Honey is a natural sweet substance that is secreted by honeybees and released into the nature of the plants' nectar or released into the secretions of some of the insects that some of them feed on live parts (Codex Alimentarius Commission Standards, 2001). Honey is generally composed of carbohydrates, nitrogenous compounds such as amino acids, organic acids, minerals, pigments, flavoring substances, vitamins, waxy substances, pollen grains and water. These compounds are parameters affecting honey viscosity, hygroscopicity, crystallization, color, flavor, density and shelf life (Ajlouni and Sujirapinyokul, 2010). Honey also contains many other substances such as Maillard reaction products, volatile compounds and bioactive substances that affect the sensory and physical traits of honey as well as biological potential. It is a complex, concentrated aqueous sugar solution that plays a crucial role in this composition, including its geographical origin, climate, botanical source, processing and storage conditions (Manzanares et al., 2011). In addition, honey is known for its natural antioxidant source and antimicrobial properties. The antioxidant capacity of honey is widely used to determine the radical sweeping ability by the widely used DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The ability of the honey material to sweep the radical properties was found to be related to the total phenolic substance myrites. Components that enhance the antioxidant traits of honey, including catalase, ascorbic acid, proxidase, carnaenoids, flavanoids, amino acids, phenolic substances, Maillard reaction products and others. It is important to note that in honey composition there is very little glucose and sucrose as sugars (Ajlouni and Sujirapinyokul, 2010).

Hydroxy methyl furfural (HMF) is naturally produced by heat treatment in fresh products, because HMF is a sign of heat-treated honey. The long-term heating of honey causes enzyme loss and fructose breakdown to form HMF. Heating honey for a long time at high temperature or storing it in bad conditions for a long period of time causes to decrease of nutrient contents and to increase amount of HMF. For this reason, increasing HMF amount is undesirable in honey. For example, HMF in fresh honey should be less than 15 mg/kg. The European Union has an HMF limit of 40 mg (Codex Alimentarius Commission Standards, 2001).

In recent years, the antibiotic properties of honey is used in cancer cases, diminished immune system, heart diseases, eye diseases such as cataracts, various injuries, and healing of inflammatory cells (Anonymous, 2002). Turkey has geographically and climatic conditions to provide a favorable environment for the development of beekeeping and honey production. Recently, a number of studies have been published related to different geographical and botanical origins of honey, physical and chemical characteristics and to determine the quality of honey. However, there is no publication on the physicochemical properties and in determining antioxidant activities of Anzer honey, which is known to be legendary and good for many diseases. The main aim of this study is to contribute to the knowledge of eastern black sea Anzer honey with different floras of eastern black sea by analyzing physicochemical properties.

MATERIALS AND METHODS

Honey samples

Anzer honey samples (~ 1000 g) were collected from the beekeepers in the Eastern Black Sea Region, Anzer Plateau Rize (altitude 40° 35' 21"N, longitude 40° 31' 0" E, altitude 2200 m). Samples were obtained in November. The quality criteria in the honey samples were analyzed in the Food Technology Department of the School of Applied Sciences Korkut Ata University and at the Ministry of Food, Agriculture and Livestock laboratories. Honey samples placed in sterilized glass containers and stored at 3°C then were analyzed within 48-36 hours after harvest. pH, moisture, total acidity, fructose, glucose, sucrose, ash, potassium, calcium, magnesium, sodium, HMF, diastase enzyme, electrical conductivity, color, TPC, TAC were investigated in honey samples. Analyzes were performed in triplicate and the methods applied were described below.

Physicochemical properties

For the determination of pH value, the probe of the pH-meter (HANNA H1 2211-02, Romania) was dipped into the honey solution prepared with distilled water (10% w/v) (Bogdanov and Edder, 2004). The refractometric method was used to measure the moisture content of honey (the honey-soluble solid which is defined by the International Honey Commission). For this purpose, the fracture index at 20°C was read using an Abbott-type refractometer (Bausch and Lomb, USA) (Anonymous, 2006c). The acidity value of the honey was determined by three-stage titration (free, lactic and total acidity value). The pH solution was added to a solution of 0.5 M NaOH that had been spun to pH 8.50. The amount of consumed hydrochloric acid solution (mL) was then recorded for titration while lowering to pH 8.30 with 0.5 M Hydrochloric acid solution. The result was calculated as meq/kg with the aid of the following equation (Bogdanov et al., 1997). Fructose, glucose and sucrose content of the major sugars contained in honey were determined by HPLC method (Anonymous, 1997). The HPLC conditions are as follows: HPLC: AGILENT 1100A, Detector: Refractive Index (RID, 1100A), Column: 250x4.6 mm ID, amine modified silica gel having a particle diameter of 5-7 µm (MACHEREY- NAGEL GmbH & Co.KG., Germany), Mobile phase: Acetonitrile: Water (4:1), Column and detector temperature: 30°C, Pump flow rate: 1.3 mL/min, Injection volume: 10 µL. 2.5 g of honey was dissolved in a 50 mL balloon flask with some water. 12.5 mL of methanol was added and 50 mL of pure water was added. The solution was filtered through a 0.45 µm membrane and then injected into HPLC. Honey quantities were calculated by comparing the peak times and peak times of the sugars in the standard solution (Anonymous, 1997). For ash analysis approximately 2.5 g of honey is weighed and is placed on a heater until the moisture fully evaporated, and then when the sample rich a certain weight placed in an ash furnace set at 600°C. The total amount of ash was calculated as percentage by weight (Anonymous, 2006b). Analysis of mineral elements (K, Ca, Na, Mg) VARIAN brand, OPTIMA 2100 model ICP-OES system was used for the analysis conditions were followed (Anonymous, 1998). Power: 1,00 kW, Pump speed: 15 rpm, Plasma flow: 15 L/min, Auxilary flow: 1.5 L/min, Nebulizer flow: 0.90 L/min. The absorbance values of the elements were measured at the following wave lengths: Potassium 405.7 nm, Calcium 397.8 nm, Sodium 589.9 nm, Magnesium 280.2 nm. 0.5 grams of honey was weighed into high pressure resistant teflon containers, 7.5 mL of 65% nitric acid was added, the mouths of the containers were tightly closed and the MARS HP500 PLUS VESSELS CEM model heat and a pressure-controlled microwave combustion unit. The HPLC method was used for HMF determination (Anonymous, 2002a). The analysis conditions by HPLC were as follows: HPLC: Aggilent 1100A, Detector: DAD (1100A), Column: 250x4.6 mm C18 column (Macherey GmbH & Co. Germany), Mobile phase: Methanol: Water (10:90), Column and detector temperature: 30°C, Pump flow rate: 1 mL/min, Injection volume: 20 µL, Wave Length: 285 nm. For analysis of honey sample, 5 g honey sample was weighed in a 100 mL balloon jug with 0.01 precision. Distillate was dissolved with water and completed to the balloon line. The solution was filtered through a 0.45 µm membrane and then injected into the HPLC system. The amount of HMF in the honey was calculated as mg/kg using peak area and standard curve. For determination of diastase, approximately 10 g of honey was dissolved in 40 to 50 mL of distilled water in a beaker. The mixture was taken into a 100 mL beaker and diluted with distilled water to the marking line. Solvents used to prepare the buffer solution to be used in the analysis are: Citric acid monohydrate solvent: 21.01 g citric acid monohydrate ($C_6H_8O_7.H_2O$) is dissolved in about 500 mL of water in a 1000 mL measuring flask and dissolved in distilled water to 1000 mL. Disodium hydrogen phosphate dehydrate solvent: 35.60 g of disodium hydrogen phosphate dehydrate ($Na_2HPO_4.2H_2O$) was dissolved in about 500 mL of water in a 1000 mL measuring flask and dissolved in distilled water to 1000 mL. Preparation of phosphate / citrate buffer: Citric acid monohydrate solution 469 mL was placed in a 2 L volumetric flask and mixed with 531 mL of the disodium hydrogen phosphate dehydrate solution. The beaker was placed on a magnetic

stirrer and the electrode was immersed in the solution after calibration of the pH meter was performed. The pH of the mixture was adjusted to pH 5.2 by titration with 0.5 N hydrochloric acid solution if the pH of the mixture was 5.2 and pH 5.2 with 0.5 N sodium hydroxide solution. Starch solution: 1 g starch is weighed into a 100 mL balloon jug and mixed with some distilled water were mixed and boiled. Once complete dissolution was achieved, it was cooled down to the marking line (Anonymous, 2002). The analysis of the determination of the electrical conductivity, which is defined as the ability of the honey to transmit electricity, is mainly based on the measurement of the electrical resistance. The instrument was calibrated with special calibration solutions. Calibration solutions used for this purpose are 0.05 % NaCl, 0.05 % KCl, 1 % NaCl, 1% KCl solutions. For measurement of the electrical conductivity of the sample, 10 g of honey was weighed in a 50 mL beaker. After dissolving with some distilled water, the balloon was taken out and 50 mL was added. About 20 mL of this solution was taken and the temperature was adjusted to 20°C and measured with a conductor meter (RADIOMETER brand, CDM 80 model). The result is given in ms/cm (Anonymous, 2002b). The color of the honey samples was measured using a Minolta colorimeter CM-2600d instrument with L *(100: white, 0: black), a * (+: red, - green) and b * (+: yellow, -: blue). The instrument was calibrated using a white tile (L* =92.48, a* = -0.24 and b* = 1.01) as standard. The results were expressed in accordance with the CIELAB system with reference to illuminant D65 and with a visual angle of 10° (Anupama et al., 2002).

Bioactive properties

The phenolic compounds, which are the basis of the total phenolic assay, are based on the redox reaction in which the Folin-Ciocalteu reagent is reduced in the basic medium and converted to its oxidized form. By measuring the absorbance of the blue color formed by the reduced reagent in the reaction result, it is possible to calculate the total amounts of the phenolic compounds to be analyzed. The color intensity of the resulting complex is directly proportional to the concentration of the phenolic materials. 2 g of honey sample was diluted with 40 mL of pure water, filtered by Whatman filter (4 times folded). Then added 1 mL of this solution, 2.5 mL of 0.2 N Folin-Ciocalteu reagent and 2 mL of 0.7 M sodium carbonate (Na_2CO_3), then stirred for 6 minutes. It was allowed to stand in the dark for 2 hours at 25 °C. After reaction, absorbance values were read at 740 nm. The total phenolic material was expressed as gallic acid equivalents (GAE) per gram of honey (Singleton and Rossi, 1965).

The antioxidant properties of honey samples were determined by the DPPH method (Socha et al., 2009). 25 g of honey were dissolved in 50.0 mL of purified water and 1 mL of this solution was added to 29 mL of DPPH solution (1: 100 methyl alcohol 80 % v / v). The sample solution and a DPPH control solution were left in the dark for 30 minutes at 25°C. The absorbance of the sample solution and a DPPH control solution was measured at 515 nm with a UV-Vis spectrophotometer (OPTIMA brand SP-3000 NANO, Japan).

Statistical analyses

Statistical analysis of the samples was performed using the SPSS 16.0 statistical program analysis (ANOVA). The differences between the samples were compared using Duncan multiple comparison tests.

RESULTS AND DISCUSSION

Physicochemical properties

Honey is a natural product with low pH and is not suitable for the growth of microorganisms. The pH refers to the presence of hydrogen ions in honey. In addition, the presence of hydrogen ions in honey is an effective parameter on texturing, composition and shelf life, which is a useful variable to estimate the quality of the product (Bogdanov et al., 1997). The pH values of the honey samples ranged from 3.74 to 4.08 (Table 1). Average the lowest pH value was found in sample 3, and the highest average value was found in sample 1 of honey. In a study on nectar, secretory and mixed honey samples in Spain, the pH value was determined between 3.29 and 4.88 (Sanz et al., 2005). In another study conducted in Algeria

and Spain, the pH value was between 3.50 and 4.58 (Rashed and Soltan, 2004). (Yılmaz and Küfrevioğlu, 2001) found that the average pH value of honey samples collected from Eastern and Southeastern Anatolia regions is 3.8. Turkish Food Codex Regulation no limit for pH is specified in Honey Communiqué, it is stated that the pH of honey should be between 3.4 and 6.1 in Turkish Standards Institute Honey Standard. The pH values found in this study are similar to other studies. In general, a low pH of honey is not suitable for the growth of microorganisms. Honey acidity is responsible for the aroma and stability against microbial deterioration. It has also been observed that honey has played an important role in the healing of the wound when it has been applied to the wound area for years. Because injuries do not heal easily usually have an alkaline environment and healing is easier in an acid environment (Gethin et al., 2008).

Table 1. Physico-chemical parameters of analyzed Anzer honey samples.

Honey samples	Moisture %	Ash %	Electrical conductivity (EC) mS/cm	pH	Total acidity (meq/kg)	HMF mg/kg	Diastase	Fructose	Glucose	Sucrose
AH1	21	0.6	0.26	4.08	9.98	1.82	38.41	30.52	18.12	0
AH2	19	0.59	0.39	3.78	15.56	3.45	36.81	31.52	17.52	0
AH3	16	0.61	0.43	3.74	9.74	4.56	35.14	32.65	17.23	0
AH4	17	0.65	0.49	3.85	17.95	5.41	35.67	33.36	19.47	0
AH5	17	0.67	0.36	4.01	21.65	3.12	36.82	33.65	20.41	0
AH6	19	0.68	0.38	4.07	25.65	2.34	37.98	32.52	21.42	0
AH7	16	0.61	0.67	3.89	9.65	5.86	34.92	36.52	34.65	0
AH8	16	0.66	0.76	3.99	14.32	6.11	33.21	38.65	34.41	0
AH9	17	0.74	0.72	3.94	13.12	12.15	21.45	40.98	36.47	0
AH10	17	0.64	0.59	4.01	15.74	11.56	23.65	32.41	25.41	0
AH11	16	0.66	0.68	4.05	14.54	8.87	31.85	34.52	28.47	0
AH12	17	0.65	0.74	4.04	19.87	7.16	34.14	35.61	33.54	0
AH13	16	0.62	0.44	4.01	18.97	9.65	29.14	30.54	21.74	0
AH14	18	0.65	0.68	3.96	21.65	8.45	29.54	31.14	22.74	0
AH15	19	0.71	0.45	4.07	25.64	3.65	37.47	31.41	23.78	0
AH16	15	0.74	0.69	3.78	27.96	10.65	19.45	30.65	19.41	0
AH17	17	0.75	0.52	3.79	20.32	12.32	20.47	30.41	18.75	0
AH18	18	0.78	0.56	3.99	12.65	17.8	9.45	31.54	19.56	0
AH19	16	0.68	0.69	3.98	11.21	15.63	10.52	30.74	22.63	0
AH20	14	0.62	0.78	4.06	17.68	15.54	9.42	39.54	24.23	0
AH21	16	0.67	0.79	3.89	16.31	8.62	28.74	40.74	33.87	0
Mean	17	0.67	0.57	3.95	17.15	8.32	28.3	33.79	24.47	0
SD	3.54	0.05	0.16	0.11	5.37	4.64	9.69	3.52	6.41	0
Min	14	0.59	0.26	3.74	9.65	1.82	9.42	30.41	17.23	0
Max	21	0.78	0.79	4.08	27.96	17.8	38.41	40.98	36.47	0

On honey moisture content, flower source, climate factors, honey harvest time, honey maturity grade, bee species play an important role. Water affects the viscosity, specific weight, maturation, crystallization, taste, preservation, shelf life and taste of honey in the content. Moisture analysis is carried out to determine the product safety at the end of the storage period to

the last point reached by the honey from acceptance of honey. It is also an important quality criterion for the possibility of fermentation of the product. Fermentation does not start when the sugars, which provide the typical sweetness and usefulness of honey, are as high in concentration as possible. When the water content is 18.5% and higher fermentation can occur. The moisture shows that the honey does not mature, or that there is water from the outside. This, in turn, promotes the development of honey, osmophilic microorganisms, resulting in the danger of surface fermentation. In addition, it affects the sensory and nutritional properties of honey in a negative way and reduces the product shelf life. The moisture content (%) in honey samples examined ranged between 14-21%. All other samples, except the AH1 honey sample (21%), have a maximum moisture content of less than 20% recommended for the International Honey Commission (Acquarone et al., 2007). One of the important quality criteria of honey is the degree of acidity. The main factors determining the acidity of honey are organic acids and minerals as well as amino acids, peptides and carbohydrates. The total acidity of the honey is 9.65 to 27.96 meq/kg (%) in the twenty-one case of honey. (Al-Doghairi et al., 2007) found a total acidity range of 9.12-93.02 meq/kg for the Saudi honey. The amount of free acid should not exceed 50 meq per 1000 g of water according to the Turkish Food Codex (TGK), Codex Alimentarius Standard Honey Commission (CAC) and European Union (EU) standards. Batu et al., (2013) reported total acidity values of 6.73-47.06 meq/kg. Velioğlu and Köse, (1983) determined the total amount of acidity in sunflower honey as 14.35 meq/kg on average. Thus, the analyzed samples are in accordance with the standards and similar to the studies done. In twenty-one Anzer honey samples, the percentages of fructose and glucose ranged from 30.41% to 40.98% and from 17.23% to 36.47%, respectively. The twenty-one studied the dominant sugar, fructose and then glucose. The high amount of sucrose in honey indicates that honey is harvested early and that sucrose is not sufficiently reduced, that the beans are fed with sucrose or that direct sucrose is added to honey (Aydın et al., 2008). No sucrose was observed in all samples. Because in Anzer's production, the bee is never given sugar. As can be seen in the sugar composition, Anzer is a proof that honey does not contain table sugar (sucrose). The maximum limit for honey samples by the European Community of sucrose is 5% (Anonymous, 2001). In addition, the fructose / glucose ratio for honey is important, and it tells us that honey is liquid when the crystallinity of honey is higher than that of glucose (Feás et al., 2010). All of the Anzer honey samples examined were in liquid state. Ash content can be used to determine the botanical origin (floral, mix or honeydew) (White et al., 1963). In the samples, the content of ash was between 0.59% and 0.78%, and the results are within the limits given for flower honey (0.6%). The minerals found in the soil can first be transferred to the plant matter and then to the honey component together with the nectar (Gul, 2008). Mineral content is one of the parameters used to evaluate nutritional values of honey. It can be thought of as a potential indicator of the geographical origins of honey and as an important biomarker for the environmental pollution of heavy metals. This analysis is important for beekeepers and consumers because it helps avoid potential contamination during processing and ensures that the product is of good quality. The concentration of mineral components in the honey varies between 0.1% and 1.0%. When compared with nectar honey, the rate of mineral matter is higher due to their high electrical conductivity (Lachman et al., 2007). The most common macro element in the honey samples taken from the Rize province in the Eastern Black Sea region was potassium (K) and the other macro elements were calcium (Ca), sodium (Na) and magnesium (Mg) and apart from these, the other elements in the honey are in the group of micro elements. Generally, the most abundant elements found in the investigated honey samples were potassium, calcium sodium, and magnesium which ranged between 1265.87 and 5887.65, 299.56 and 854.36, 289.41 and 591.45, and 41.54 and 90.54 mg/kg honey, respectively. Variability in the individual amounts of minerals in analyzed honey samples can be attributed to differences in flower type and soil composition (table 2) (Solayman, et al., 2016).

Table 2. Minerals of analyzed Anzer honey samples.

Honey samples	Potassium	Calsium	Sodium	Magnesium
AH1	1265.87	299.56	289.41	41.54
AH2	1386.23	300.78	294.41	45.65
AH3	1452.32	301.75	301.74	48.65
AH4	1657.52	301.47	302.47	48.62
AH5	1956.87	302.52	307.65	49.41
AH6	2652.14	312.42	385.47	52.47
AH7	3256.74	684.14	547.68	65.74
AH8	4625.98	768.23	557.87	78.41
AH9	5468.23	798.65	561.24	69.74
AH10	2365.87	402.87	398.47	55.84
AH11	4020.35	704.89	524.65	66.87
AH12	4374.21	735.41	536.41	68.79
AH13	1657.51	400.65	397.54	55.47
AH14	3024.87	627.54	498.65	56.87
AH15	1469.85	325.45	389.47	54.65
AH16	3542.65	697.31	538.97	79.47
AH17	2147.63	311.41	384.54	53.21
AH18	2687.32	598.25	532.14	89.87
AH19	4217.41	728.69	565.14	84.54
AH20	5662.37	823.65	589.21	88.65
AH21	5887.65	854.36	591.45	90.54
Mean	3084.74	537.14	445.16	64.05
SD	3268.09	392.3	213.57	34.65
Min	1265.87	299.56	289.41	41.54
Max	5887.65	854.36	591.45	90.54

Heat treatment is usually applied to prevent the tendency of the honey to crystallize, to remove the crystal appearance from the center and to neutralize the honey-infecting microorganisms. Depending on the temperature and duration, honey heat treatment may decrease the amount of vitamins, nutrients and diastase activity in honey and increase the amount of HMF. For this reason, the HMF content of a honey sample gives information about the temperature grade of the applied process. However, in this regard, the chemical composition of honey must also be taken into account (Kambur et al., 2015). HMF is usually not found in newly produced honey, and increases with time due to conditions and storage (Chakir et al., 2011). HMF is a substance which has harmful effects on human health, resulting in the storage of carbohydrates or storage in environments that are not heat-stable. Pehlivan and Gül, (2015) reported HMF levels of 12.8 mg /kg and 20.32 mg/kg in the case of Euphorbia echinus and Euphorbia resinifera honey and 30.43 mg/kg in thyme honey. In general, the HMF content of the honey samples of the Anzer honey samples varied between 1.82-17.80 mg/kg. All samples were tested for the highest value of 40 mg/kg, which was determined for the Turkish Food Codex Honey Communiqué and the European Union standards for HMF value and they are found to be in conformity with the aforementioned standards for HMF by the European Community regulations (Feás et al., 2015). Diastase is an enzyme naturally found in the structure of honey. The amount in the honey may vary depending on the geographical and origin of the flora. On the other hand, the freshness of the honey also affects the number of diastases in the honey. Along with HMF, diastase

activity has been exposed to heat and can be used as an indicator of long-standing happiness (Karadal and Yıldırım, 2012). It has been reported that the source of diastase and invertase enzymes as a common result of the studies made is the salivary secretions of each of these (Won et al., 2009). The diastase number is a quality parameter and is used to determine whether honey has been subjected to heat treatment until packaged and delivered to the consumer. Still heat treatment is usually applied to prevent the tendency of the honey to crystallize, to remove the crystal appearance from the surface, to neutralize the microorganisms and to reduce the viscosity. However, depending on the thermal treatment applied to the honey, the quality of the wax results in loss of quality due to the temperature and duration of application, causing the diastase activity to decrease and the amount of HMF to increase (Karadal and Yıldırım, 2012). In general, the diastase of the Anzer honey samples varied between 9.42-38.41 mg/kg. Electrical conductivity (EC) varies depending on the proportion of honey, especially minerals, total ash, salts, proteins and acid. As the mineral and acid ratio of honey increased, the electrical conductivity increased accordingly (Da Silva et al., 2016). The results obtained after examining twenty-one honey samples show that the EC values are between 0.26-0.79 mS/cm. Thus, this parameter is a good criterion for the identification of honey floras, the definition of flower honey and honey bees (Lazarević et al., 2012). In the other two studies, electrical conductivity values of flower honey samples were determined as 0.69 mS/cm and 0.19 mS/cm, respectively (Fallico et al., 2004). It was determined that the conductance values we determined in our study were partially close to those. According to Turkish Food Codex Honey Communiqué and European Union standards, flower honey electrical conductivity should be lower than 0,8 mS/cm (Anonymous, 2005). In this study, it was determined that the honey samples are suitable to these standards. One of the most important factors affecting the visuality of honey is the color. The materials responsible for the honey color are not exactly known, but the color of the honey, can vary from white to dark brown depending on the plant source, storage period and conditions (Krell, 1996). Honey color is associated with the amount of phenolic acids, chlorophyll, carotene and xanthophyll protonocyanidins and flavonoids and minerals present in the honey (González-Paramás et al., 2007). Many studies have shown that the amount of antioxidant is higher in dark colored honey than in light colored honey (Pontis et al., 2014). Color values of the honeys are given in Figure 1. Color values of honeys are expressed as L*/ for darkness/lightness (0 black, 100 white), a*/ (−a greenness, +a redness), and b*/ (−b blueness, +b yellowness). Honey color is mainly composed of total mineral (ash) content, and light colored honey is generally associated with low ash content. Honey color depends on the flora involved and on associated vitamin, pigment, phenolic substance, mineral contents, pollen color, nonenzymatic browning reactions and the waiting period after harvest. Comparing our results with values in the previous literature, the samples exhibited lower L values, defined as dark-color honeys. Light-colored honeys are reported to have L values lower than 50, and our L values AH1, AH2, AH7, AH8, AH9, AH10 and AH20 were also below 50. Dark-colored honeys are reported to have L values higher than 50, and our L values AH3, AH4, AH5, AH6, AH11, AH12, AH13, AH14, AH15, AH16, AH17, AH18, AH19 and AH21 were also above 50. Dark-colored honeys are reported to have a stronger taste, while light-colored honeys have a delicate flavor (Nombré et al., 2016). The phenolic content of Anzer honey and the difference in color may be indicative of the presence of different nectar resources in the surrounding area, rather than the single source of nectar.

Bioactive properties

Among the components responsible for antioxidant, antimicrobial and antiinflammatory properties of honey are phenolic compounds (Kolayli et al., 2016). In this study, total phenolic content (TPC) of honey was determined by the method of Folin-Ciocalteu. The results of the total phenolic content analysis are shown in Figure 2. The lowest TPC value was 802.6 μg GAE/g honey in AH_6 and the highest TPC value was 1352, 6 μg GAE/g honey in AH_1. In a study

by Noor et al., (2014) the total amount of phenolic substances in natural and commercial honey is 36.01-252.00 and 1.33-140.55 GAE mg/100g, respectively; Wilczyńska, (2014) reported 40.5-177 mg GAE/100 g. Silva et al., (2013b) reported to be 31,91 mg GAE/100g. Compared with the results obtained from our study, it was concluded that the phenolic content of the honey samples is quite high.

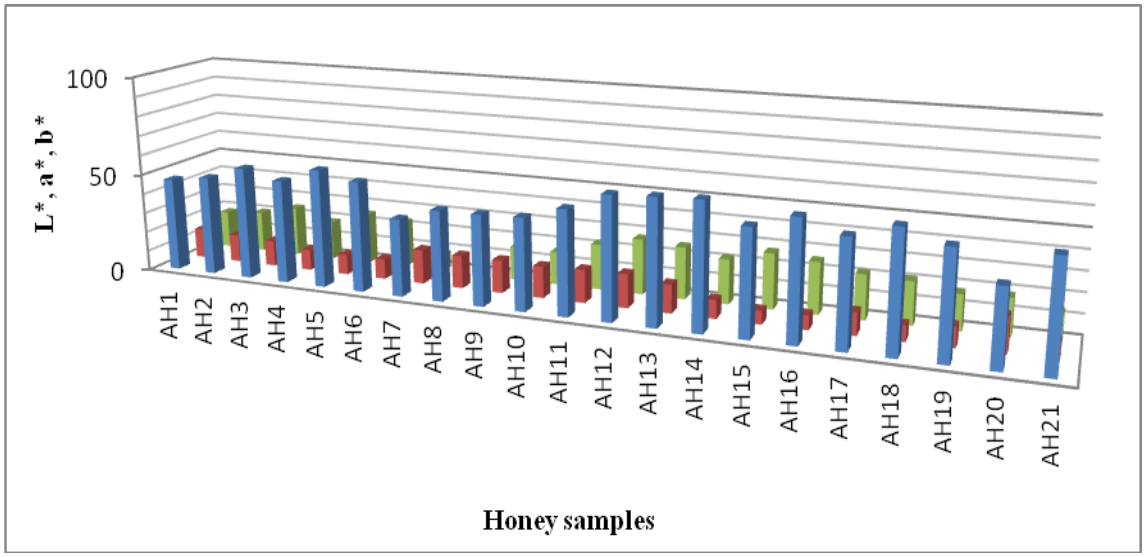

Figure 1 . Color of honey samples

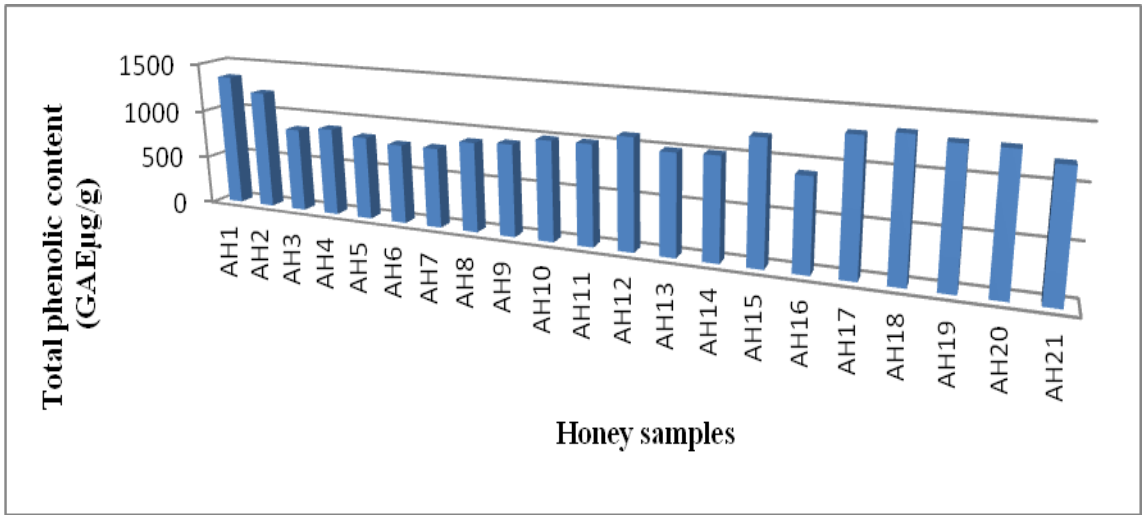

Figure 2 . Total phenolic content of honey samples (GAEμg/g)

Antioxidants present naturally in honey; they exhibit antioxidant effects through free radical linkers, reducing agents, metal chelators, or singlet oxygen scavenging mechanisms and have a positive effect on metabolism. The DPPH method was used to determine the antioxidant content of the samples. According to this, the antioxidant content of honey samples ranged from 9.12-20.69 μmol TE/g (Figure 3). The highest value is 20.69 μmol TE / g in AH_1 and the lowest value is 9.12 μmol TE / g in AH_6. Gasic et al., (2014) found the amount of antioxidants in Serbian honey samples to be 1.31-25.61 μmol TE /g by DPPH method. Gethin et al., (2008) found

that the highest antioxidant activity was 1.22 µmol TE / g in the highest water and 0.48 µmol TE / g in the lowest pineapple. Halim et al., (2011) reported an antioxidant value of 0.38-0.59 µmol TE/g in samples of Indian honey specimens. The antioxidant content results of the present study samples are generally found higher than above studies.

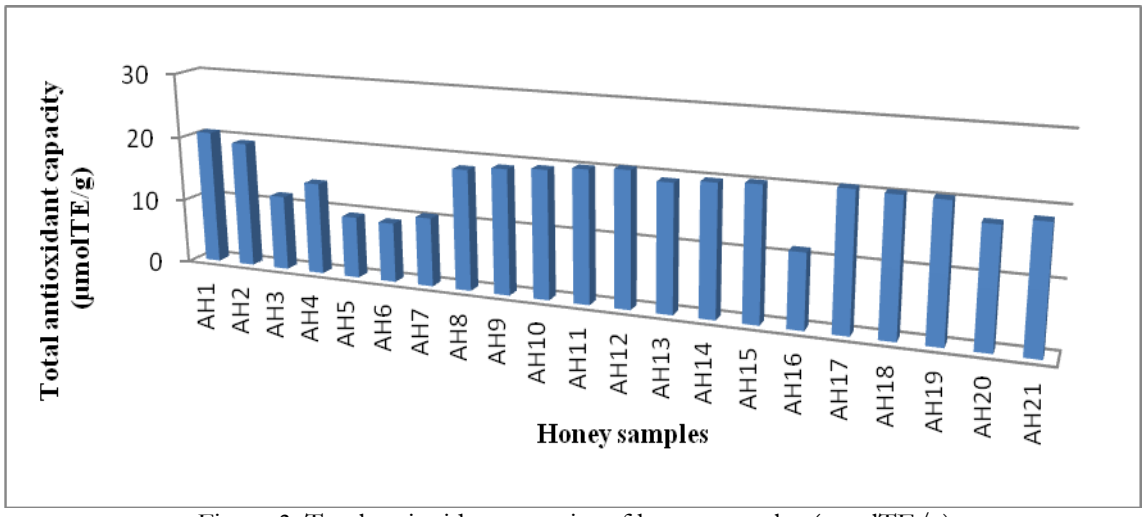

Figure 3. Total antioxidant capacity of honey samples (µmolTE/g)

CONCLUSION

In this study, twenty-one samples of the Anzer honey were investigated for their physicochemical properties such as pH, electrical conductivity, fructose, glucose composition and HMF. The obtained data allows us to evaluate the quality of twenty-one over-all honey samples and to create some rules that emphasize their qualities. The results also show that twenty-one Anzer honey samples are characterized by total phenolic content (TPC) and total antioxidant content (TAC). It is believed that these components can provide great economic and/or industrial benefits due to applications in the food, cosmetic and pharmaceutical industries. In the end, honey is a natural product with a number of distinctive therapeutic properties. However, it is suggested that more elaborated studies reveal other hidden features of Anzer honey. Knowing the boundaries of reference values for biochemical and physicochemical values on a regional basis is of great importance in terms of reaching the information needed for product export. Results of this study should be confirmed by similar study results of which are due to the fact that the plant richness of Eastern Black Sea region to determine the standard reference values that represents these regions.

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