KSÜ Tarım ve Doğa Derg 21(6):936-943, 2018
KSU J. Agric Nat 21(6):936-943, 2018

Examination of Some Biochemical Properties of Honeys in Mutki (Bitlis-Turkey)

Ayşe Dilek ÖZŞAHİN KİREÇCİ[1] Oğuz Ayhan KIREÇCİ[2],
[1]Bitlis Eren University, Faculty of Science and Arts, Bitlis, [2]Bitlis Eren University, Hizan Vocational School, Bitlis, Turkey
✉: molekuler@gmail.com

ABSTRACT
In this study, the sugar content, total phenolic content, flavonoid types and levels of hydroxymethylfurfural (HMF), proline of 13 honey samples collected from Bitlis-Mutki were analyzed. The Mutki district is at 1500 altitude and has terrestrial climate characteristics. In winter there is heavy snowfall and the summers are hot and dry. According to the sugar analysis of honey samples, the samples contained arabinose, fructose, glucose, sucrose and maltose. Glucose and fructose were found at the maximum amount, but sucrose at the minimum level in the samples. In addition, two comparative antioxidant assays, namely DPPH and ABTS radicals scavenging assay, were applied to detect the antioxidant power of honey samples. The antioxidant and chemical properties in Bitlis-Mutki's honeys make it to be high added value and excellent quality product. In conclusion, it was observed that the results were in accordance with the EU standards as well as the Turkish Food Codex Honey Notification.

DOI:10.18016/ksutarimdoga.vi.423630

Article History
Received : 15.05.2018
Accepted : 16.07.2018

Keywords
Antioxidant capacity,
Flavonoid,
HMF,
Mutki honey,
Sugar

Research Article

Mutki (Bitlis-Türkiye) Ballarının Bazı Biyokimyasal Özelliklerinin Araştırılması

ÖZET
Bu çalışmada, Bitlis-Mutki'den toplanan 13 bal örneğinin şeker içeriği, total fenolik içeriği, flavonoid türleri ve hidroksimetilfurfural (HMF) düzeyleri ölçüldü. Mutki ilçesi 1500 rakıma sahiptir. Mutki karasal iklim özelliklerine sahiptir. Kış aylarında yoğun kar yağışı olup, yazları sıcak ve kuraktır. Bal örneklerinin şeker analizi sonuçlarında arabinoz, früktoz, glukoz, sukroz ve maltoz bulundu. Bal örneklerinde glukoz ve früktozun maksimum düzeyde, sukrozun ise minimum seviyede bulunduğu belirlendi. Buna ek olarak, bal örneklerinin metanolik ektsraklarının DPPH ve ABTS radikallerini temizleme aktiviteleride incelenmiştir. Bitlis-Mutki'nin ballarındaki antioksidan ve kimyasal özellikler, onları yüksek katma değerli ve mükemmel kalitede ürünler haline getirmektedir. Sonuç olarak, sonuçların AB standartlarına ve Türk Gıda Kodeksi Bal Tebliğine uygun olduğu görülmüştür.

Makale Tarihçesi
Geliş Tarihi: 15.05.2018
Kabul Tarihi : 16.07.2018

Anahtar Kelimeler
Antioksidan kapasite,
Flavonoid,
HMF,
Mutki balı,
Şeker

Araştırma Makalesi

To cite: Kireçci OA, Özşahin Kireçci, AD 2018. Examination of some biochemical properties of honeys in Mutki (Bitlis-Turkey). KSÜ Tar Doğa Derg 21(6) : 936-943, DOI : 10.18016/ksutarimdoga.vi.397124:

INTRODUCTION

Honey is unique product containing natural rich source of amino acids, vitamins, minerals, biologically active compounds and sweet concentrated solution of readily available sugars produced by honey bees (Nayik and Nanda, 2015). Naturally, honey has been traditionally recognized as a valuable source of energy. Being a complex food product, the composition of honey depends on not the only floral source, but also many factors geographical origin, climatic conditions, storage period, temperature as well as environmental factors (Nayik and Nanda, 2015).

Honey is known to be rich in antioxidants, including flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins (Gheldof et al., 2002; Lachman et al., 2010; Schramm et al., 2003). Honey is considered a part of apitherapy since early human, and has recently been used in treatment of burns, gastrointestinal disorders, chronic wounds, asthma, skin ulcers, cataracts etc. due to its antimicrobial, antioxidant, antiviral, anti-inflammatory, anticancer and immunosuppressive activities (Subrahmanyam et al., 2001; Kucuk et al., 2007). Both animal studies and clinical trials in different

parts of the world revealed some highly promising results regarding the healing potential of honey (Subrahmanyam et al., 2001; Kucuk et al., 2007; Nayik et al., 2015). The Mutki district is at 1500 altitude and has terrestrial climate characteristics. In winter there is heavy snowfall and the summers are hot and dry. In the district, there are 10.500 hives and thus Mutki can produce about 150 tons of honey per year. The land structure consists of mountainous, wooded, steep valleys and hills. The general plant cover of the town is forest (about 40%), agricultural land (about 10%), and remaining being meadow, pasture, steppe and rocky.

Due to features such as its geographical position, climatic conditions and three seasons of the year being suited to honey production, Turkey is one of the richest regions of the world in terms of honey production and floral variety. It is home to a wide variety of nectar and honeydew honey types, both monofloral and multifloral. The purpose of this study was to reveal the chemical characterizations and antioxidant activities of honey samples from Mutki (Bitlis-Turkey) and to identify honeys with a high apitherapy potential for future studies. So far, no research has been conducted to determine the total phenolic, sugar content, flavonoid types, levels of HMF with prolin and antiradical scavenging of Mutki region honeys. In the present study, we investigated the above mentioned constituents of honey samples collected from thirteen different regions of Mutki.

MATERIAL and METHODS

Honey Materials

Thirteen varieties of honey samples were collected from Mutki-Bitlis of Turkey (harvested in 2015) through the producers. Fresh honey samples were weighted as 250 g, packed and sealed in glass bottles, were purchased from local producers and stored at 4°C. The samples were analyzed as soon as they were received in the laboratory. None of the samples exceeded the storage period beyond six months. The honey samples were kept at ambient temperature (25 ±2°C) overnight before the analyses.

Chromatographic conditions for flavonoid analysis

Initially, 1 g of a sample was dissolved in 10 mL of 80% methanol, homogenized by centrifuging at 5,000 rpm for 5 min at 4°C and filtered through a filter paper. Chromatographic analysis was carried out using PREVAIL C18 reversed-phase column (15 × 4.6 mm) 5 µm diameter particles. The mobile phase was methanol/water/acetonitrile (46/46/8, v/v/v) containing 1.0% acetic acid (Zu et al., 2006). The mobile phase was filtered through 0.45 µm membrane filters (Millipore), then diaurated ultrasonically prior to use. Catechin, naringin, rutin, myricetin, morin and quercetin were quantified by DAD detector following RP-HPLC separation at 280 nm for catechin and naringin, 254 nm for rutin, myricetin, morin and quercetin. The flow rate and injection volumes were 1.0 mL/min and l0 µL, respectively. The chromatographic peaks of the analyses were confirmed by comparing their retention time and UV spectra with those of reference standards. Quantification was carried out by peak area integration using the external standard method. All chromatographic operations were carried out at an ambient temperature of 25°C.

Determination of sugars using liquid chromatography

10 g of fresh honey samples were homogenized in 100 mL of distilled water. Homogenates were centrifuged at 5,000 rpm for 5 min at 4ºC and the supernatant collected. Sugar concentrations in the combined extracts were determined using high performance liquid chromatography (HPLC) with a refractive index detector (RID). The mobile phase was acetonitrile/water (75/25, v/v) and elution was performed at a flow-rate of 1 mL/min, at 40°C constant column temperature (Ozsahin and Yılmaz, 2010). The column used was a supelcosil-NH2, (25 × 4.6 mm, 5 µm, Sigma, USA). The analyses were performed in triplicate batches. Prior to the quantitative and qualitative determination of sugars in the sample, standard solutions of different sugars were prepared: sucrose, maltose, glucose and fructose. These standard solutions were used in the preparation of calibration curves for each corresponding sugar and used for assessing the concentrations corresponding to the different peaks in the chromatograms.

Antioxidant assay by DPPH radical scavenging activity

The 2 g of honey sample was dissolved in 10 mL of distilled water. Homogenates were centrifuged at 5,000 rpm for 5 min at 4ºC and filtered through a filter paper. The free radical scavenging activities of the extract was assessed by the discoloration of the methanolic DPPH· solution according to the method of Brand-Williams et al., (1995). A solution of 25 mg/L DPPH in methanol was prepared and 4.0 mL of this solution was mixed with 100 and 250 µL of the extract in DMSO. The reaction mixture was stored in darkness at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical scavenging activity (%) = [(Abs control–Abs sample)]/(Abs control)] × 100 where Abs control is the absorbance of DPPH radical + methanol; Abs sample is the absorbance of DPPH radical + sample extract/standard.

ABTS–+ cation radical scavenging

The ABTS test was performed according to the methodology by Re et al., (1999). The cation radical ABTS–+ was synthesized by the reaction of a 7 mM ABTS solution with a 2.45 mM potassium persulfate solution. The mixture was kept at 23°C in the dark room for 16 h. Afterwards, the ABTS–+ solution was diluted with ethanol until an absorbance (A) of 0.7 at 734 nm was

reached in a UV–VIS spectrophotometer. Aliquots of 2.7 mL from the $ABTS^{\cdot+}$ solution were added, immediately after being prepared, to the sample solutions diluted in methanol to reach final concentration range of 0.1 and 0.5 mg/-mL. After 10 min, the percent inhibition of absorbance at 734 nm was calculated for each concentration, relative to blank. The scavenging capability of the $ABTS^{\cdot+}$ radical (%AS) was calculated using the following equation:

$$\% AS = 100(A_{control} - A_{sample}) / A_{control}$$

where $A_{control}$ is the control absorbance obtained from the $ABTS^{\cdot+}$ radical alcoholic solution, and A_{sample} is the radical absorbance in the presence of the sample or the Trolox standard.

Total phenolic content

The total phenolic content was determined by the Folin–Ciocalteu method (Singleton et al., 1999). Thirty microlitres of honey solution (0.1 g/mL) were mixed with 2.37 mL of milli Q water and 150 ll of 0.2 N Folin–Ciocalteu reagent. The solution was thoroughly mixed by vortexing and incubated for 2 min at room temperature. Four hundred and fifty microlitres of sodium carbonate solution (0.2 g/mL) were added to the reaction mixture and further incubated for 2 h at ambient temperature. The absorbance was measured at 765 nm using a spectrophotometer (Agilent Cary 60 Uv Vis G6860 A). The total phenolic content was determined by comparing with a standard curve prepared using gallic acid (0–200 mg/L). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

Determination of 5-hydroxymethylfurfural (HMF) content

5-HMF contents of the honey samples were determined according to the modified method of Zappalà et al., (2005). Ten grams of honey sample was diluted with the addition of 40 mL distilled water, filtered with 0.22 µm filter and injected to an HPLC (Shimadzu, Kyoto, Japan). C18 column, 5 µm and 4.6 × 250 mm, was used for 5-HMF analyze. The mobile phase, flow rate and injection volume were 95% acetic acid solution (1%) and 5% acetonitrile, 1 mL/min and 20 µL, respectively. The wavelength of the detector was set at 284 nm. The 5-HMF concentration was determined by using a standard calibration curve.

Proline content

The proline content was determined based on its reaction with ninhydrin which forms a colored complex. After adding 2-propanol, the absorbance of the sample solution and a reference solution at 510 nm using spectrophotometer was determined. Results were expressed in proline milligrams per kilograms of honey (Codex Alimentarius Commission 2001).

Statistical analysis

One-way analysis of variance (ANOVA) and Post Hoc Tukey-HSD test were used to determine differences between groups. Results are presented as mean ± S.E.M. Values were considered statistically significant if $P<0.05$. The SPSS/PC program (Version 15.0; SPSS, Chicago, IL) was used for the statistical analysis.

RESULTS

Flavonoids, Total Phenolic, Hydroxymethylfurfural (HMF) and Proline Contents of Mutki Honeys

Flavonoid analysis determined that catechin, morin and naringenin were present in all honey samples (Table 1). Comparison of catechin contents showed that the H1 group had the highest content whereas other H8 and H13 groups were present at lowe levels. Among all the honey groups, the morin amount was highest in the H13 group whereas other H8 and H13 groups were present at lower levels. In honey samples; H2 and H12 groups contain a high level of naringenin flavonoid, although no significant difference was other groups (Table 1). These results demonstrate the variability in the quantities compounds based on honey type. When levels of total phenolics and total flavonoid were compared, it was found that the H6 group contained high levels of phenolic and flavonoid content, although no significant differences were found in the other groups (Table 1).

Comparison of HMF contents showed that the H3 and H9 groups ($p<0.0001$) has the lowest content whereas no difference was observed among the other groups (Table 1). When proline quantities are compared between groups; it was observed in all samples that this amino acid was present at certain ratios, but in the samples H7 and H8 there was proline at a significantly lower level ($p<0.001$; $p<0.0001$).

Sugar Contents of Mutki Honeys

Sugar analysis showed that arabinose, fructose, glucose and maltose were present in all honey samples (Table 2). When the amounts of sugars were compared, it was found that glucose and fructose were the most high sugar types. Comparison of arabinose contents showed that the H7 group had the highest content whereas other H1 and H12 groups were present at lower levels. It was also observed that fructose content was high in the H13 group, but not statistically significantly different among other groups (Table 2). Fructose is always quantitatively the most important sugar, followed by glucose. In the current study, glucose was lower than fructose in all the honey samples analyzed. When glucose contents were compared it was found glucose amount was significantly high in the H13 group and that there was no difference among honey groups. Statistical comparison of maltose contents indicated that H2, H5 and H8 groups contained significantly low amounts of this sugar and there was no significant difference between other groups. Among all the honey groups, the

sucrose amounts were highest in the H12 group whereas no difference was observed among the other groups (Table 2).

Table 1: Flavonoids, Total Phenolic, and Proline Contents of Mutki Honeys

Honey Samples N=3	Catechin (µg/g)	Morin (µg/g)	Naringenin (µg/g)	Total Phenolic Content (µg/100 g)	HMF (µg)	Proline (mg/g)
Honey 1	389.83±0.15	3.05±0.02	12.22±0.09	17.82±0.2	0.20±0.03	1.55±0.10
Honey 2	356.27±0.23	4.77±0.01	2.77±0.06	23.85±0.6	0.20±0.02	1.35±0.09
Honey 3	190.27±0.36	7.88±0.05	1.22±0.01	19.33±0.1	0.07±0.00^{cd}	1.38±0.10
Honey 4	333.05±0.11	2.72±0.03	1.27±0.05	21.63±0.3	0.33±0.05	1.41±0.12
Honey 5	319.55±0.27	3.38±0.06	1.61±0.07	38.80±0.1	0.13±0.02	1.51±0.11
Honey 6	373.11±0.12	2.77±0.05	1.27±0.02	51.55±0.9	0.20±0.01	1.25±0.09
Honey 7	230.27±0.29	0.38±0.08	0.55±0.05	33.98±0.2	0.20±0.02	0.95±0.09^{d}
Honey 8	146.50±0.22	0.61±0.02	0.50±0.01	21.28±0.3	0.27±0.04	0.87±0.08^{cd}
Honey 9	198.05±0.38	7.83±0.03	1.27±0.06	30.15±0.6	0.07±0.00^{cd}	1.43±0.12
Honey 10	-	1.77±0.07	1.38±0.09	39.07±0.6	0.13±0.02	1.54±0.14
Honey 11	-	2.88±0.08	1.33±0.03	25.97±0.9	0.13±0.02	1.61±0.11
Honey 12	174.05±0.09	4.83±0.03	2.44±0.08	36.22±0.3	0.13±0.02	1.20±0.09
Honey 13	139.22±0.87	9.61±0.06	1.27±0.08	28.20±0.2	0.13±0.01	1.52±0.10
General average	219.24±9.97	4.04±0.44	2.24±0.22	29.83±0.09	0.17±0.01	1.35±0.78

cd: p<0.0001

Table 2: Sugar Content of Tested Honeys (mg/10 g)

Honey Samples N=3	Arabinose	Fructose	Glucose	Sucrose	Maltose
Honey 1	13.85±0.04	349.46±0.25	253.97±0.11	13.83±0.03	10.80±0.04
Honey 2	19.93±0.06	343.18±0.31	246.60±0.15	15.97±0.05	8.95±0.04
Honey 3	24.41±0.05	344.31±0.18	247.04±0.12	10.62±0.02	10.04±0.02
Honey 4	22.12±0.06	353.84±0.26	245.23±0.17	14.60±0.06	13.98±0.05
Honey 5	27.34±0.03	336.90±0.20	243.89±0.15	18.40±0.06	8.50±0.02
Honey 6	28.40±0.05	342.28±0.15	241.23±0.09	15.64±0.04	11.18±0.06
Honey 7	177.86±0.15^{cd}	329.35±0.11	239.03±0.10	12.97±0.02	10.25±0.05
Honey 8	22.02±0.02	342.84±0.32	241.87±0.11	13.92±0.03	8.76±0.02
Honey 9	24.93±0.03	317.65±0.21	237.99±0.09	10.57±0.02	15.45±0.07^{cd}
Honey 10	27.73±0.03	347.12±0.25	250±0.12	16.19±0.07	9.63±0.03
Honey 11	19±0.02	346.49±0.30	256.84±0.16	15.71±0.05	14.60±0.06^{cd}
Honey 12	11.37±0.04	338.71±0.27	252.24±0.20	25.75±0.09^{d}	10.35±0.03
Honey 13	17.75±0.08	357.58±0.33	263.04±0.25	14.73±0.02	12.50±0.05
General Avarage	33.59±0.45	342.29±0.89	247.61±0.77	15.30±0.12	11.15±0.16

cd: p<0.0001

Antioxidant Capacity of Mutki Honey Samples

In the present study, ABTS and DPPH assays were preferred to evaluate the antioxidant activities of honey samples from Mutki region. According to the results of DPPH free radical scavenging activity, all samples displayed antioxidant activity from 100 µl concentration (Table 3). The H11 had more significant radical cleaning characteristics than the other groups in 100 µl concentration. When the groups were compared depending on increasing concentration the group having the highest antioxidant capacity was the H6 group. When the radical scavenging activity of honey samples was compared at the interval of 250 µl concentration, it was detected that the H6 group was more effective than other concentrations. The H6 and H12 groups were found to have more significant radical cleaning characteristics than the other groups in 250 µl concentration (Table 3). The ABTS assay is one of the most frequently used analytical strategies for antioxidant activity. The results of the determination of ABTS free radical scavenging are demonstrated in Table 3. The lowest radical scavenging activity was determined for sample H9. When the groups were compared depending on increasing concentration the group having the highest antioxidant capacity was the H5 group (Table 3).

Table 3: DPPH and ABTS radical scavenging effects of honey samples (%)

Honey Samples	DPPH (100 µL)	DPPH (250 µL)	ABTS (100 µL)	ABTS (250 µL)
Honey 1	80.06±0.09	69.37±0.06	82.27±0.05	59.53±0.02
Honey 2	69.78±0.02	74.39±0.03	61.14±0.09	69.56±0.06
Honey 3	38.16±0.1	45.87±0.21	47.90±0,11	63.02±0.12
Honey 4	79.60±0.06	78.12±0.02	70.28±0,06	60.52±0.05
Honey 5	76.95±0.09	75.85±0.24	66.52±0.06	82.45±0.07
Honey 6	76.48±0.03	85.09±0.02	82.63±0.03	61.68±0.02
Honey 7	41.74±0.11	53.48±0.13	56.85±0.27	69.83±0.07
Honey 8	64.33±0.09	72.77±0.06	65.17±0.07	71.44±0.06
Honey 9	24.45±0.02	32.90±0.09	33.12±0.03	54.88±0.04
Honey 10	81.31±0.08	75.36±0.04	77.08±0.08	67.68±0.05
Honey 11	80.53±0.17	76.50±0.05	59.00±0.21	74.40±0.05
Honey 12	72.43±0.07	79.42±0.04	61.77±0.08	70.10±0.02
Honey 13	44.86±0.06	52.84±0.01	61.24±0.02	70.72±0.07
General Avarage	63.9	67.07	63.46	67.37

DISCUSSION

Honey is produced and largely used as an important energy food and medicinal source (El-Haskoury et al., 2017). Honey is a highly supersaturated solution of a complex mixture of sugars, it also contains a small amount of other constituents such as minerals, proteins, vitamins, organic acids, flavonoids, phenolic compounds (Sime et al., 2015). The determination of phenols, flavones and flavonols contents is an important criterion for determining the nutritional quality of honey; this composition depends on the botanical origin of honey (Fernandez-Torres et al., 2005; Habib et al., 2014; Escriche et al., 2014). Our results showed the richness of the all samples honey for these bioactive molecules. The phenolic content of our honey samples was similar to those reported in Yemen and Morocco honeys (75.13-246.21 mg/100 g) (Al-Mamary et al., 2002) and was higher than that obtained in from Brazil (61.16-111.37 mg GAE/100g) (Bueno-Costa et al., 2016), India (49-98 mg GAE/100 g) (Saxena et al., 2010), Serbia (27.44-61.42 mg GAE/100 g) and Algeria (15.84-61-63 mg/10 g) (Mouhoubi-Tafinine et al., 2016).

Sugar is the main components of honey which depend mostly on floral and geographical origins and less on seasonal, processing and storage conditions (Ouchemoukh et al., 2010; Dobre et al., 2012). Sugar composition has been used to discriminate honey samples on the basis of floral as well as the geographical origin (Gomez-Barez et al., 2000). In the study, sugar analysis showed that arabinose, fructose, glucose and maltose were present in all honey samples. Glucose and fructose were the major carbohydrates of all honey samples analyzed. Sucrose contents of the honey samples were in the range of 10.57 and 25.75 mg/100 g. None of the samples exceeded the European Codex Honey Standards, which is 5 g/100 g for honeys (Codex Alimentarius, 2001). A higher sucrose content observed in one honey sample can be attributed to reasons such as overfeeding of honeybees with sucrose syrup, adulteration, or an early harvesting of honey, where sucrose has not been fully transformed into glucose (Anklam 1998; Saxena et al., 2010). In addition, 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 (Kucuk et al., 2007, Tornuk et al., 2013). Generally, the sugar composition of the honey is affected by the plant species flowers used by the bees, regional and climatic conditions (Mateo and Bosch-Reig, 1998). 5-HMF is a good indicator for honey freshness and/or overheating.

5-HMF can form at even low temperatures in acidic conditions by dehydration of sugar (Lee and Nagy, 1990). However, 5-HMF alone can not be used for the determination of the severity of the heat treatment. As the temperature of the thermal treatment and storage increase, its concentration increases drastically (Capuano and Fogliano, 2011). It was reported that there was not a direct relationship between the 5-HMF content of honey and its composition (Turhan et al., 2008). This statement may be true in the case of that only one factor such as sucrose concentration is considered and other factors such as pH, fructose and glucose concentration, phenolic contents of the honey are eliminated. However, several factors influence the levels of HMF, such as temperature and time of heating, storage conditions, pH and floral source, thus it provides an indication of overheating and storage in poor conditions (Fallico et al., 2006). Overall, 5- HMF content of honeys should be below 40 mg/kg according to the International Standards of Codex Alimentarius (2001). In the present study, the HMF of the thirteen examined Mutki honeys ranged from 0.07 to 0.20 µg/g. All honey samples had an HMF value lower than the above limit, and none showed values higher than 40 mg/kg.

Honey contains 20 amino acids with proline being the

highest quantities present. The amount of proline in honey is a marker of purity and its level decreases significantly in adulterated honeys. The level of proline has been reported to vary according to the honey flora, but this is more closely associated with the bees' work performance (Cotte et al., 2004; Can et al., 2015,). Experimental studies have reported that honey from bees fed on sugar water exhibit low proline values (Cavrar et al., 2013). According to honey codices, the desired level of proline in honey is 250 mg/kg, although the level is much higher in quality honeys and the codies need to be revised (Codex, Standard 2001). The proline amount for the honey samples used in our study varied between the interval 0.87 mg/g- 1.61 mg/g. When the results from our study were compared; it can be stated that the proline amount of the honey samples we used were quite high and that they were very nutritious in terms of protein.

Several studies demonstrate that a great number of medicinal and aromatic herbs, as well as fruits and leaves of some plants, biosynthesize phytochemicals possessing antioxidant activity and may be used as a natural source of free radical scavenging compounds (Jawanmardi et al., 2002). The majority of these plants are used by the bees to collect honey nectar; consequently, plant origin bioactive components can be transferred to honey (Baltrusaityte et al., 2007). Recentely, honey is often investigated in terms of antioxidant power as an eligible parameter for quality due to acceptance as a natural antioxidant (Lachman et al., 2007). In the present study demonstrates that the DPPH and ABTS radicals scavenging activity of Mutki honeys are significantly high in all honey samples. Phenolic compounds play a major role in the antioxidant activity of natural products. The differences between honey samples in terms of antioxidant activity could be attributed to the natural variations in floral sources of nectar and the different locations. Although honey by itself may not serve as a major source of dietary antioxidants, it demonstrates the potential to play a role in providing antioxidants in a highly palatable form.

CONCLUSION

In conclusion, this study presents the results of a wide spectrum of biochemical parameters including flavonoid, total phenolic, proline and HMF contents, antioxidant capacity and sugar profiles obtained by the analysis of Bitlis-Mutki honeys originating from Turkey. Mutki honeys, contain important total phenolic concentrations, showing a substantial antioxidant capacity which may be used as a natural source of compounds with these properties. This study is the first report about the phytochemical composition, HMF and proline analysis, the antioxidant activity of Mutki region honeys. The higher total phenolic content honey samples were determined to have a high radical scavenging property. These results support the hypothesis that phenolic compounds contribute greatly to the nutritional value of honey. Therefore, more investigations are highly recommended to elucidate the potential use of honey as a rich source of natural antioxidant phenolics. Our data is of particular interest in defining the effect of botanical origin in the biological activity of honey and to confirm its importance on the availability of phytochemistry compounds.

ACKNOWLEDGEMENTS

The authors are also would like to thank experienced beekeepers Mr. Alican Yasar, Soner Altepe, Adil Ozalp, Sabri Konut, Hüsamettin Kartal, Felemez Bulac, Resul Dursun, Ramazan Kardas, İsmail Balci, Besim Akdas, İrfan Bilit and Erkan Dogan, who supplied the honey samples.

REFERENCES

Al-Mamary A, Al-Meeri M, Al-Habori M 2002. Antioxidant activities and total phenolics of different types of honeys. Nutritions Research, 22: 1041-1047. http://dx.doi.org/10.1016/S0271-5317(02)00406-2

Anklam E 1998. A review of the analytical methods to determine the geographical and botanical origin of honey. Food Chemistry, 63: 549–562. https://doi.org/10.1016/S0308-8146(98)00057-0

Baltrušaityte V, Venskutoris PR, C˘eksteryte V 2007. Radical scavenging activity of different floral origin honey and beebread phenolic extracts. Food Chemistry, 101: 502–514. doi: http://dx.doi.org/10.1016%2Fj.foodchem.2006.02.007

Brand-Williams W, Cuvelier ME, Berset C 1995. Use of free radical method to evaluation of antioxidant activity. LWT – Food Science and Technology, 28(1): 25–30.

Bueno-Costa FM, Zambiazi RC, Bohmer BW, Chaves FC, Padilha Da Silva W, Zanusso JT, Dutra I 2016. Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. LWT-Food Science and Technology, 65: 333-340. https://doi.org/10.1016/j.lwt.2015.08.018

Can Z, Yildiz O, Sahin H, Akyuz Turumtay E. Silici S, Kolayli S 2015. An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chemistry, 180: 133-141. https://doi.org/10.1016/j.foodchem.2015.02.024.

Canadanovic-Brunet J, Cetkovic G, Saponjac VT, Stajcic S, Vulic J, Stajner D, Popovic B 2014. Evaluation of phenolic content, antioxidant activity and sensory characteristics of Serbian honey-based product. Industrial Crops and Products, 62: 1-7. https://doi.org/10.1016/j.indcrop.2014.08.009

Capuano E, Fogliano V 2011. Acrylamide and 5-Hydroxymethylfurfural (HMF): a review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT – Food Science and Technology, 44: 793–810. https://doi.org/10.1016/j.lwt.2010.11.002.

Cavrar S, Yildiz O, Sahin H, Karahalil F, Kolayli S 2013. Comparison of physical and biochemical characteristics of different quality of Turkish honey. Uludag Bee Journal, 13: 55-62.

Codex Alimentarius Commission. 2001. Revised standards for honey. Codex Standard 12-1981. Rev 1 (1987), Rev 2 (2001), Rome, FAO.

Cotte JF, Casabianca H, Giroud B, Albert M, Lheritier J, Grenier-Loustalot MF 2004. Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. Analytical and Bioanalytical Chemistry, 378: 1342-1350. https://doi.org/10.1007/s00216-003-2430-z

Djilas S, Lachman J, Orsak M, Hejtmankova A, Kovarova E 2010. Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT-Food Science and Technology, 43: 52-58. https://doi.org/10.1016/j.lwt.2009.06.008

Dobre I, Georgescu LA, Alexe P, Escuredo O, Seijo MC 2012. Rheological behavior of different honey types from Romania. Food Research International, 49: 126-132. https://doi.org/10.1016/j.foodres.2012.08.009

El-Haskoury R, Kriaa W, Lyoussi B, Makni M 2018. Ceratonia siliqua honeys from Morocco: Physicochemical properties, mineral contents and antioxidant activities. Journal of Food and Drug Analysis, 26(1): 67-73. https://doi.org/10.1016/j.jfda.2016.11.016

Escriche I, Kadar M, Juan-Borras M, Domenech E 2014. Suitability of antioxidant capacity, flavonoids and phenolic acids for floral authentication of honey. Impact of industrial thermal treatment. Food Chemistry, 142: 135-143. http://doi.org/10.1016/j.foodchem.2013.07.033

Fallico B, Arena E, Verzera A, Zappala M 2006. The european food legislation and ıts ımpact on honey sector. Accreditation and Quality Assurance, 11: 49–54. http://dx.doi.org/10.1007/s00769-006-0128-6

Fernandez-Torres R, Perez-Bernal JL, Bello-Lopez MA, Gallejon-Mochon M, Jimenez-Sanchez JG, Guiraum-Perez A 2005. Mineral content and botanical origin of Spanish honeys. Talanta, 65: 686-691. doi: 10.1016/j.talanta.2004.07.030.

Gheldof N, Wang XH, Engeseth NJ 2002. Identification and quantification of antioxidant components of honeys from various floral sources. Journal of Agricultural and Food Chemistry, 50: 5870-5877. https://doi.org/10.1021/jf02561135.

Gomez-Barez JA, Garcia-Vilanova RJ, Elvira-Garcia S, RivasPala T, Gonzalez-Paramas AM, Sanchez-Sanchez J 2000. Geographical discrimination of honeys through the employment of sugar patterns and common chemical quality parameters. European Food Research Technology, 210: 437–444. http://dx.doi.org/10.1007/s002170050578

Habib HM, Al Meqbali FT, Kamal H, Souka UD, Ibrahim WH 2014. Physicochemical and biochemical properties of honeys from arid regions. Food Chemistry, 153: 35-43. https://doi.org/10.1016/j.foodchem.2013.12.048.

Jawanmardi J, Khaligh A, Kash, A, Bais HP, Vivanco JM 2002. Chemical characterization of basil (*Ocimum basillicum* L.) found in local accessions and used in traditional medicines in Iran. Journal of Agricultural and Food Chemistry, 50(21): 5878–5883. https://doi.org/10.1021/jf020487q.

Kucuk M, Kolayli S, Karaoglu S, Ulusoy E, Baltaci C, Candan F 2007. Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chemistry, 100: 526-534. https://doi.org/10.1016/j.foodchem.2005.10.010

Lachman J, Kolihova D, Miholova D, Košata J, Titera D, Kult K 2007. analysis of minority honey components: possible use for the evaluation of honey quality. Food Chemistry, 101(3): 973–979. https://doi.org/10.1016/j.foodchem.2006.02.049

Lee HS, Nagy S 1990. Relative reactivities of sugars in the formation of 5-hydroxymethyl furfural in sugar-catalyst model systems. Journal of Food Processing and Preservation, 14: 171–178. https://doi.org/10.1111/j.1745-4549.1990.tb00126.x.

Mateo R, Bosch-Reig F 1997. Sugar profiles of spanish unifloral honeys. Food Chemistry, 60(1): 33–41.

Mouhoubi-Tafinine Z, Ouchemoukh S, Tamendjari A. 2016. Antioxydant activity of some algerian honey and propolis. Industrial Crops and Products, 88: 85-90. http://dx.doi.org/10.1016/j.indcrop.2016.02.033

Nayik GA, Nanda V 2015. Physico-chemical, enzymatic, mineral and colour characterization of three different varieties of honeys from Kashmir valley of India with a multivariate approach. Polish Journal of Food and Nutrition Sciences, 65(2): 101-108. https://doi.org/10.1515/pjfns-2015-0022

Ouchemoukh S, Schweitzer P, Bachir Bey M, Djoudad-Kadji H 2010. HPLC sugar profiles of Algerian honeys. Food Chemistry, 121: 561-568. https://doi.org/10.1016/j.foodchem.2009.12.047

Ozsahin AD, Yilmaz O 2010. *Prunus armeniaca* L. cv. hacihaliloglu fruits extracts prevent lipid peroxidation and protect the unsaturated fatty acids in the fenton reagent environment. Asian Journal of Chemistry, 22: 8022–8032.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C 1999. Antioxidant activity applying an ımproved abts radical cation decolorization assay. Free Radicals and Biology Medicine, 26: 1231–1237. https://doi.org/10.1016/S08915849(98)00315-3

Saxena S, Gautam S, Sharma A 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chemistry, 118: 391-397. https://doi.org/10.1016/j.foodchem.2009.05.001

Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M, Keen CL 2003. Honey with high levels of antioxidants can provide protection to healthy human subjects. Journal of Agricultural and Food Chemistry, 51: 1732-1735. https://doi.org/10.1021/jf025928k.

Sime D, Atlabachew M, Redi-Abshiro M, Zewde T 2015. Total phenols and antioxidant activities of natural honeys and propolis collected from different geographical regions of Ethiopia. Bulletin of the Chemical Society of Ethiopia, 29(2): 163-172. http://dx.doi.org/10.4314/bcse.v29i2.1

Singleton VL, Orthofer R, Lamuela-Raventos RM 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin–ciocalteu reagent. Methods in Enzymology, 299: 265–275. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

Subrahmanyam M, Sahapure AG, Nagane NS, Bhagwat VR, Ganu JV 2001. Effects of topicalapplication of honey on burn wound healing. Annals of Burns and Fire Disasters, 14: 143-145.

Tornuk F, Karaman S, Ozturk I, Toker OS, Tastemur B, Sagdic O, Dogan M, Kayaciger A 2013. Quality characterization of artisanal and retail turkish blossom honeys: determination of physicochemical, microbiological, bioactive properties and aroma profile. Industrial Crops and Products, 46: 124-131. doi: http://dx.doi.org/10.1016/2Fj.indcrop.2012.12.042

Turhan I, Tetik N, Karahan M, Gurel F, Tavukcuoglu R 2008. Quality of honeys ınfluenced by thermal treatment. LWT – Food Science and Technology, 41: 1396–1399. doi: http://dx.doi.org/10.1016%2Fj.lwt.2007.09.008

Yucel Y, Sultanoglu P 2013. Characterization of honeys from Hatay region by their physicochemical properties combined with chemometrics. Food Bioscience, 1: 16-25. https://doi.org/10.1016/j.fbio.2013.02.001

Zappalà M, Fallico B, Arena E, Verzera A 2005. Methods of the determination of 5-hmf in honey: a comparison. *Food Control* 16: 273–277. https://doi.org/10.1016/j.foodcont.2004.03.006.

Zu Y, Li C, Fu Y, Zhao C 2006. Simultaneous determination of catechin, rutin, quercetin kaempferol and isorhamnetin in the extract of sea buckthorn (*Hippophae rhamnoides* L.) leaves by RP-HPLC with DAD. Journal of Pharmaceutical and Biomedical Analysis, 41: 714–719. . https://doi.org/10.1016/j.jpba.2005.04.052