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**PJBS**

ISSN 1028-8880

**Pakistan  
Journal of Biological Sciences**

**ANSI***net*

Asian Network for Scientific Information  
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

## Biological Activities and Some Physicochemical Properties of Sunflower Honeys Collected from the Thrace Region of Turkey

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**Abstract:** Honey is a sweet food made by bees using nectar from flowers. Its quality depends on a number of factors, such as floral type, pH, moisture, free acidity, diastase activity, invert sugar and sucrose. The aim of the study is to examine the qualities of 50 sunflower honey (*Helianthus annuus* L.) collected from the Thrace region of Turkey, in terms of melissopalynological analysis, important chemical parameters and antioxidant activities. The total phenolic content of the honey samples was determined by the Folin-Ciocalteu method with spectrophotometry. The 1,2-diphenyl-2-picryl hydrazyl (DPPH) method was used to determine anti-radical activity and the phosphomolybdenum method was utilized for antioxidant activity. Correlations between the analysed parameters were found to be statistically significant ( $p < 0.05$ ). The results obtained for physicochemical characteristics of sunflower honey indicate a good quality level, adequate processing, good maturity and freshness and that the sunflower honey samples studied proved to be good source of natural dietary antioxidants. This is the first report of the total phenolic content, antioxidant and antiradical activities of sunflower honeys collected from the Thrace region of Turkey.

**Key words:** Sunflower honey, physico-chemical parameters, antioxidant, antiradical, phenolic content

### INTRODUCTION

Honey is the natural sweet product produced by *Apis mellifera* bees from nectar of plants (nectar honey), a highly concentrated solution of a complex mixture of carbohydrates. The monosaccharides, fructose and glucose, are the main sugars found in honey (Nagai *et al.*, 2002; Ouchemoukh *et al.*, 2007). Besides this, it also contains certain minor constituents viz., proteins, enzymes (invertase, glucose oxidase, catalase, phosphatases), amino and organic acids (gluconic acid, acetic acid, etc.), lipids, vitamins (ascorbic acid, niacin, pyridoxine etc.), volatile chemicals, phenolic acids, flavonoids and carotenoid like substances and minerals (Saxena *et al.*, 2010). As a natural, unprocessed and easily digested food, honey can be regarded as an important part of our diet (Feas *et al.*, 2010).

Since, the 1970s researchers from different scientific fields have investigated the chemical and biological properties of honey but only recently has there been an increased interest in application of antioxidants to the medical treatment of different diseases caused by oxidative stress (Aljadi and Kamaruddin, 2004; Beretta *et al.*, 2005; Al *et al.*, 2009). Honey is a sweet and flavorful natural product which has been consumed for its high nutritive value and its contribution in human health. It was demonstrated that honey on a fresh weight

basis is similar to many fruits and vegetables in its antioxidant capacity (Gheldof and Engeseth, 2002). Honey contains a variety of phenolics and represents a good source of antioxidants which makes it a good food antioxidant additive and increases its usability potential in ethnomedicine (Aljadi and Kamaruddin, 2004; Al-Mamary *et al.*, 2002; Beretta *et al.*, 2005). Honey contains a number of components known to act as antioxidants; these include vitamin C, vitamin E, enzymes such as catalase, peroxidase and phenolic compounds (Aljadi and Kamaruddin, 2004). In the recent years there has been an increasing interest in determination of the antioxidant activity of honey. Many studies indicated that the antioxidant activity of honey varies widely, depending on the floral source (Alvarez-Suarez *et al.*, 2010). The botanical origin of honey has the greatest influence on its antioxidant activity while processing, handling and storage affect honey antioxidant activity only to a minor degree (Al-Mamary *et al.*, 2002; Beretta *et al.*, 2005; Lachman *et al.*, 2010; Baltrusaityte *et al.*, 2007; Gheldof and Engeseth, 2002).

Honey is produced in almost every country of the world and it is very important energy food. Honey cannot be considered a complete food by human nutritional standards but it does offer potential as a dietary supplement (Mendes *et al.*, 1998). Honey is reported to contain about 200 substances and is considered as an important part of traditional medicine (Ferreira *et al.*, 2009).

In Turkey, thanks to geographical and climatic conditions that provide a suitable environment for apiculture, honey production has been well developed. The beekeeping that has been sustained in Turkey for thousands of years is an important agricultural activity. (Kahraman *et al.*, 2010). Turkey is one of the top honey producers in the world with bee colonies numbering to about 4.4 million and 70 000 tons of honey produced annually (Soysal and Gurcan, 2005). Honey is mainly produced in the central and western regions of Turkey. The Thrace region of Turkey which is a major sunflower producing area, raises the potential of the area as a possible sunflower honey producer (Yardibi and Gumus, 2010).

Sunflower honey is bright yellow, smells fragrant, dry, with an aroma of pollen, slightly herbaceous. It has a lively, pleasant taste and is often called the traditional honey. Sunflower honey always has a creamy quality and a fine texture which is easy to spread. Sunflower honey crystallizes quickly and looks like a candle.

It has been reported some the physico-chemical features of honey produced in the Edirne province of Thrace region. Correlations between all the analyzed parameters are evaluated. Until now, there has been no research to determine the total phenolic content, antioxidant and antiradical activities of sunflower honeys collected from the Thrace region of Turkey. In the present study, it has been investigated the above mentioned parameters of fifty sunflower honeys.

## MATERIALS AND METHODS

**Sunflower honey samples:** Fifty sunflower honey samples were obtained from different beekeepers in various regions of the Edirne province in Turkey. The samples were collected in 200 g glass bottles and immediately transferred to the laboratory and kept at 4-5°C.

**Pollen analysis:** The 50 honey samples were classified according to their botanical origin using the methods of Louveaux *et al.* (1978). The aim of that analysis was to confirm that the analyzed samples could be declared as heather monofloral honey. Briefly, pollen analyses are based on the extraction of pollen grains from 10 g of crude honey. The sample was dissolved in distilled water and the sediment was concentrated by repeated centrifuging 15' at 3000 rpm. The prepared pollen preparations were examined under the microscope. They were described and also compared with the pollen atlas. The number of dominant pollens was determined. The following terms were used for pollen frequency classes: predominant pollen (more than 45% of pollen grains counted), secondary pollen (16-45%) and important minor pollen (3-15%).

**Physicochemical parameters:** The samples of honey were analyzed by TSE (Institute of Turkish Standards) (Anonymous, 2002a). Moisture in honey was determined in a refractometer (Anonymous 2002b). To determine acidity, 10 g of the honey samples was carefully dissolved in 75 mL CO<sub>2</sub> free distilled water and titrated with 0.1 N NaOH and the pH of the honey solution was measured by a pH meter. The diastase activity, invert sugar and sucrose were determined according to Anonymous (2002a). To determine diastase activity: a fixed amount of honey and a fixed concentration of starch solution were kept at a constant temperature by mixing. Starch in the honey was hydrolyzed due to the effect of the enzyme diastase. The terms and duration of the experiment indicated that after hydrolysis [not the rest of the hydrolysis of starch, iodine solution was transformed into the complex by a treatment with a color]. Starch solutions of different volumes were exposed to the same process and then, the starch solution that is completely able hydrolysis 1 g of honey was calculated. Invert sugars were determined using Fehling's solution by titration at boiling point against a solution of reducing sugars in honey using methylene blue as an internal indicator. The difference in concentrations of invert sugar before and after the hydrolysis procedure (inversion) was multiplied by 0.95 to achieve the apparent sucrose (AS) content.

**Determination of total phenolic content:** The total phenolic contents of the samples were determined with Folin-Ciocalteu reagent according to the method of Singleton and Rossi Jr. (1965). Each honey sample (1 g) was dissolved in 4 mL of methanol using a vortex mixer. The solution was filtered through Whatman No. 1. Briefly, 40 µL of the methanol solution of the extract (1 mg mL<sup>-1</sup>) was mixed with 2400 µL of distilled water. 200 µL non-diluted Folin-Ciocalteu reagents, 600 µL sodium carbonate (20% Na<sub>2</sub>CO<sub>3</sub>) and 760 µL distilled water were then added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 765 nm against a methanol blank and compared to a Gallic acid calibration curve. The data were presented as the average of triplicate analyses. Results were calculated as mg Gallic acid equivalents (GAE)/100 g of honey.

**Evaluation of total antioxidant capacity by phosphomolybdenum method:** The antioxidant activity of the honey samples was determined by the phosphomolybdenum method according to Prieto *et al.* (1999). First, 0.4 mL of the methanolic extract (1 mg mL<sup>-1</sup>) was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Methanol was used as a blank instead of honey solution. The tubes were capped and incubated in a water bath at 95°C for 90 min. Absorbance of the green

phosphomolybdenum complex was measured at 695 nm. The data were presented as the average of triplicate analyses. Antioxidant activity was expressed as ascorbic acid equivalents (mg AAE/1 g honey).

**Determination of antiradical scavenging activity:** The radical scavenging activity of the honey samples against the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical which results in the bleaching of the purple color exhibited by the stable DPPH radical, was evaluated according to the method of Gyamfi *et al.* (1999), with some minor modifications. Each honey sample (1 g) was dissolved in 4 mL methanol using a vortex mixer and the solution was filtered through Whatman No. 1. 50 µL of the honey samples was mixed with 450 µL Tris-HCl (pH = 7.4) and 1000 µL of  $6 \times 10^{-5}$  mM DPPH in methanol. Methanol was used as a control instead of extract. The mixtures were left for 2 h at room temperature in the dark and absorbance at 517 nm was measured using methanol as a blank. The data were presented as the average of triplicate analyses. Radical scavenging activity was expressed as percentage inhibition of the DPPH radical and was calculated by the following equation:

$$\text{Antiradical activity (\%)} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

**Statistical analysis:** All chemical data were expressed as Means  $\pm$  standard Deviations (SD). One-way analysis of variance (ANOVA) followed by least significant difference (Tukey's) was used to compare the data (phenolic, antioxidant and antiradical). Differences between means at 95% ( $p < 0.05$ ) confidence level were considered statistically significant. Correlations were obtained by Pearson's correlation coefficient ( $r$ ) in bivariate linear correlations using the SPSS statistical programme.

## RESULTS AND DISCUSSION

The qualities of 50 sunflower honey (*Helianthus annuus* L.) samples from different parts of Edirne province of the Thrace region in Turkey were evaluated, in terms of melissopalynological analysis and important chemical parameters such as pH, moisture, free acidity, diastase activity, invert sugar and sucrose.

The characterizations were as follows: moisture (20.09 $\pm$ 0.68%), pH (3.87 $\pm$ 0.33), acidity (49.84 meq kg<sup>-1</sup> $\pm$ 7.73), diastase (20.37 number $\pm$ 3.82), invert sugar (110.09 $\pm$ 2.69%) and sucrose (1.31 $\pm$ 0.51%). The total phenolic content of the honey samples ranged from 6.896 $\pm$ 0.19-23.201 $\pm$ 0.79 mg GAE/100 g honey. The antioxidant activity of the honey samples was between

78.091 $\pm$ 1.68-128.673 $\pm$ 1.99 mg AAE g<sup>-1</sup> honey. The radical scavenging activities of the honey samples varied between 24.647 $\pm$ 1.01%-65.437 $\pm$ 0.44%.

**Melissopalynological analysis:** Usually, a honey is considered mainly from one plant unifloral if the pollen frequency of that plant is >45%. All of the examined honey samples predominantly contained pollen belonging to sunflower species. Sunflower pollen contents range from 45.16-70.00% and 50 samples >45 pollen of sunflower (*Helianthus annuus* L.) (Table 1).

Table 1: Geographical origin and pollen frequency (%) of honey samples

Sample No.	Geographical origin <sup>a</sup>	Sunflower pollen frequency (%)
A1	Kocahidir/Ipsala	56.03
A2	Alicopehlivan/Ipsala	53.96
A3	Yenimuhacir/Kesan	55.09
A4	Alicopehlivan/Ipsala	51.85
A5	Kocahidir/Ipsala	45.36
A6	Boztepe/Kesan	49.44
A7	Yenimuhacir/Kesan	45.93
A8	Merkez/Kesan	50.85
A9	Merkez/Kesan	54.31
A10	Karahisar/Kesan	49.81
A11	Boztepe/Kesan	56.47
A12	Boztepe/Kesan	45.31
A13	Lalacik/Keşan	52.25
A14	Karasati/Keşan	58.27
A15	Alicopehlivan/Ipsala	54.02
A16	Alicopehlivan/Ipsala	48.66
A17	Alicopehlivan/Ipsala	55.55
A18	Koyuntepe/Ipsala	53.15
A19	Yenimuhacir/Kesan	51.52
A20	Yenimuhacir/Kesan	45.65
A21	Boztepe/Kesan	54.15
A22	Alicopehlivan/Ipsala	45.16
A23	Merkez/Kesan	70.00
A24	Alicopehlivan/Ipsala	45.48
A25	Mecidiye/Kesan	47.47
A26	Merkez/Kesan	47.22
A27	Yenimuhacir/Kesan	56.41
A28	Yenimuhacir/Kesan	53.44
A29	Yenimuhacir/Kesan	46.61
A30	Yenimuhacir/Kesan	64.28
A31	Yenimuhacir/Kesan	51.79
A32	Yenimuhacir/Kesan	60.17
A33	Merkez/Kesan	51.93
A34	Merkez/Süloölu	51.96
A35	Merkez/Enez	45.83
A36	Merkez/Meric	45.94
A37	Merkez Lalapasa	54.13
A38	Merkez/Uzunköprü	51.39
A39	Merkez/Ipsala	45.47
A40	Merkez/Ipsala	60.74
A41	Merkez/Kesan	53.53
A42	Merkez/Kesan	57.35
A43	Merkez/Kesan	53.28
A44	Merkez/Havsa	51.81
A45	Merkez/Kesan	48.66
A46	Yenimuhacir/Kesan	46.23
A47	Boztepe/Kesan	47.38
A48	Merkez/Havsa	51.85
A49	Merkez Lalapasa	46.45
A50	Merkez/Uzunköprü	48.14

<sup>a</sup>All locations are related to Edirne province

Table 2: Chemical properties of sunflower honey

Sample No.	Moisture (%)	pH	Total acidity (meq kg <sup>-1</sup> )	Diastase activity	Invert sugar (%)	Sucrose (%)
A1	20.2	3.80	48.46	17.9	102.67	1.58
A2	20.2	3.79	50.81	17.9	111.73	1.03
A3	20.1	3.80	44.31	17.9	111.03	1.54
A4	20.3	3.75	53.41	17.9	110.48	1.52
A5	20.5	3.73	53.46	17.9	110.35	1.41
A6	20.4	3.78	49.26	23.0	109.97	1.20
A7	20.1	3.77	58.11	17.9	111.29	0.93
A8	19.3	3.79	48.26	17.9	110.79	1.33
A9	20.3	3.78	53.51	17.9	110.51	1.84
A10	19.2	4.45	32.30	17.9	104.62	1.84
A11	20.2	3.79	53.61	23.0	104.03	0.90
A12	20.4	3.79	54.11	23.0	110.06	1.41
A13	19.3	3.84	58.11	23.0	108.99	0.48
A14	20.1	5.90	33.61	38.5	111.50	1.77
A15	20.3	3.74	54.31	23.0	105.38	1.11
A16	20.4	3.75	48.71	23.0	111.74	1.77
A17	20.2	3.76	47.31	23.0	111.35	1.87
A18	20.1	3.76	47.61	23.0	112.22	0.83
A19	19.5	4.47	33.15	17.9	108.75	3.42
A20	20.1	4.16	40.46	23.0	111.10	1.02
A21	20.2	3.77	53.76	29.4	110.08	0.59
A22	20.1	3.73	53.91	23.0	113.04	0.95
A23	20.0	3.84	64.11	23.0	111.74	1.57
A24	20.4	3.77	53.11	23.0	110.63	1.22
A25	20.2	3.73	53.81	17.9	110.92	1.22
A26	20.4	3.77	52.86	17.9	112.72	1.27
A27	20.2	3.74	43.86	23.0	112.99	2.36
A28	20.3	3.78	47.56	17.9	110.95	1.95
A29	20.4	3.78	48.86	23.0	113.24	1.06
A30	19.5	3.70	68.12	17.9	111.82	0.72
A31	20.1	3.72	45.76	17.9	112.31	1.14
A32	20.1	3.75	72.22	23.0	111.53	1.14
A33	22.3	3.80	41.78	17.9	111.96	0.72
A34	19.2	3.76	54.41	17.9	112.61	1.27
A35	22.1	3.80	53.96	17.9	111.24	1.34
A36	19.2	3.75	48.72	17.9	103.54	0.98
A37	19.2	3.74	49.21	17.9	106.95	1.04
A38	20.4	3.78	47.26	17.9	112.11	0.62
A39	20.2	3.77	50.86	23.0	111.86	1.35
A40	19.5	3.79	54.61	17.9	106.67	0.56
A41	22.2	3.83	48.22	17.9	105.25	0.63
A42	19.5	4.18	47.35	23.0	111.80	1.35
A43	19.3	3.71	40.86	17.9	111.64	1.35
A44	20.1	3.77	44.72	17.9	106.98	2.11
A45	19.3	3.81	53.72	23.0	107.35	1.64
A46	20.2	4.21	53.85	17.9	112.64	0.83
A47	20.3	3.71	52.11	17.9	108.22	1.46
A48	19.4	4.11	47.46	17.9	110.24	1.41
A49	19.2	3.82	33.73	17.9	111.12	1.54
A50	20.1	3.75	48.51	17.9	111.91	1.46
Mean	20.09	3.87	49.84	20.37	110.09	1.31
Standard deviation	0.68	0.33	7.730	3.820	2.69	0.51
Minimum	19.2	3.70	32.30	17.90	102.67	0.48
Maximum	22.3	5.90	72.22	38.50	113.24	3.42
TSE	≤20%		≤50 (CB) <sup>1</sup> ≤50 (SB) <sup>2</sup>	≤8 (CB) <sup>1</sup> ≤8 (SB) <sup>2</sup>	≤65 (CB) <sup>1</sup> ≤45 (SB) <sup>2</sup>	≤5 (CB) <sup>1</sup> ≤10 (SB) <sup>2</sup>
KODEKS	≤20%		≤50 (CB) <sup>1</sup> ≤50 (SB) <sup>2</sup>	≤8 (CB) <sup>1</sup> ≤8 (SB) <sup>2</sup>	≤65 (CB) <sup>1</sup> ≤45 (SB) <sup>2</sup>	≤5 (CB) <sup>1</sup> ≤10 (SB) <sup>2</sup>
EU	≤20%		≤50 (CB) <sup>1</sup> ≤50 (SB) <sup>2</sup>	≤8 (CB) <sup>1</sup> ≤8 (SB) <sup>2</sup>	≤65 (CB) <sup>1</sup> ≤45 (SB) <sup>2</sup>	≤5 (CB) <sup>1</sup> ≤10 (SB) <sup>2</sup>

CB= blossom honey, SB = honey dew honey

**Physicochemical parameters:** The results of the physicochemical analyses of sunflower honeys from different parts of the Edirne province in Turkey are presented in Table 2.

The moisture content levels of the samples were in the range of 19.2-22.3% with an average of 20.09±0.68%. In this study, thirty five of the samples were higher than the TSE, Codex and EU standards in terms of

moisture content (Anonymous 2002a, b, 2012). The moisture content of honey is an important factor, contributing to its stability against fermentation and granulation during storage (Singh and Bath, 1997). High moisture content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow (Gomes *et al.*, 2010). These findings are in the close agreement with those given as 19.5, 18.19 and 18.1 by Bath and Singh (1999), Devillers *et al.* (2004) and Sahinler and Gul (2004), respectively for sunflower honey.

The pH values of the honey samples used in this study were found to be in the range of 3.70-5.90 with an average of  $3.87 \pm 0.33$ . Various researchers reported that pH had a mean of 5.6, 3.67, 3.8 and 3.888, respectively for sunflower honey (Sahinler and Gul, 2004; Bath and Singh, 1999; Oddo *et al.*, 1995; Devillers *et al.*, 2004). pH is of great importance during honey extraction and storage, due to influence on texture, stability and presentation (Ozcan *et al.*, 2006; Terrab *et al.*, 2002).

The acidity of honey is due to the presence of organic acids, particularly the gluconic acid, in equilibrium with their lactones or esters and inorganic ions such as phosphate and chloride (Al-Khalifa and Al-Arif, 1999; Silva *et al.*, 2009). In this study, the mean value of total acidity was found as  $49.84 \text{ meq kg}^{-1} \pm 7.73$  in the range of  $32.3-72.22 \text{ meq kg}^{-1}$ . Twenty nine of the samples were in an acceptable range but twenty one of the samples had a higher value than the standards. A high total acidity may mean that the honey has fermented at some time and that the resulting alcohol was converted into organic acid (Al-Khalifa and Al-Arif, 1999). Various researchers reported that total acidity had a mean of 47.32, 40.9, 23.38-34.92  $\text{meq kg}^{-1}$ , respectively (Nanda *et al.*, 2003; Sahinler and Gul, 2004; Yardibi and Gumus, 2010). Our findings were approximately similar with these results. In contrast, significantly lower levels than the data presented here were reported for sunflower honeys as 26.2 and 19.91  $\text{meq kg}^{-1}$  by Oddo *et al.* (1995) and Devillers *et al.* (2004) respectively. The variation in acidity among different honey types may be attributed to variation in these constituents due to extraction season (Nanda *et al.*, 2003).

As a result, the biochemical analysis of the honey samples it was found that diastasis activities ranged between 17.9 and 38.5 and the average number of diastases was found to be  $20.37 \pm 3.82$ . Our findings are in agreement with TSE, Codex and EU standards (Table 2). In previous studies, these results were detected as 31.4, 25.04, 15.4, 17.9, 24.1, 18.3 and 10.9 by Bath and Singh (1999), Devillers *et al.* (2004), Oddo *et al.* (1995), Sahinler and Gul (2004), Kahraman *et al.* (2010), Silva *et al.*

(2009) and Ozcan *et al.* (2006), respectively. The variation in the activity of diastases may be related to the source of honey as well as to the climate of the region (Singh and Bath, 1997).

Invert sugar (or reducing sugars), mainly fructose and glucose, have been found to be the major constituent of honey (Mendes *et al.*, 1998; Kucuk *et al.*, 2007). In this study, the mean of invert sugars was found as  $110.09 \pm 2.69\%$  with the range of 102.67-113.24%. Invert sugars in the honey samples were found to comply with the standard values. It was reported that the invert sugar % had a mean of 76.8, 69.0, 70.3, 66.8 and 71.9% by Oddo *et al.* (1995), Sahinler and Gul (2004), Yilmaz and Kufrevioglu (2000), Kucuk *et al.* (2007) and Kahraman *et al.* (2010), respectively. Invert sugar values were determined to be higher than the values obtained in the other studies; it was thought that this was caused by the storage of the honey for a long time.

A higher sucrose content observed in one sample could be attributed to reasons such as overfeeding of honeybees with sucrose syrup, adulteration, or an early harvest of honey, wherein sucrose has not been fully transformed into glucose and fructose (Anklam, 1998; Saxena *et al.*, 2010). The value of sucrose of sunflower honey samples ranged between 0.48 and 3.42 while the average was determined to be  $1.31 \pm 0.51$ . As a result of the analysis of sample values for sucrose, the TSE, CODEX and EU standards were found to be in accordance with the obtained values. Yardibi and Gumus (2010) reported sucrose content in sunflower honey samples between 1.69 and 2.39%. In another studies, these results were detected as 1.9, 3.80 and 1.8 by Sahinler and Gul (2004), Kahraman *et al.* (2010) and Yilmaz and Kufrevioglu (2000), respectively.

**Total phenolic content of sunflower honey:** The total phenolic content (mg gallic acid/100 g of honey), as estimated by the Folin-Ciocalteu reagent method, ranged from  $6.896 \pm 0.19-23.201 \pm 0.79$  gallic acid equivalent for sunflower honeys (Table 3). The statistical differences among the total phenolic contents of the sunflower honeys were important ( $p < 0.05$ ). Honey sample A37 had the lowest total phenolic content in the honey samples tested. However, A28 had the highest total phenolic content honey sample. Mostly sunflower honey because it is light colored, has a low phenolic content. Many researchers found that honeys with dark color have a higher amount of total phenolic compounds (Gheldof and Engeseth, 2002).

The total phenolic content of certain honey samples was previously determined (Aljadi and Kamaruddin, 2004; Beretta *et al.*, 2005; Ferreira *et al.*, 2009; Lachman *et al.*,

Table 3: Total phenolic content, antioxidant and antiradical activity of honey samples

Sample No.	Total phenolic content (mg GAE/100 g honey)	Antioxidant activities of samples (mg AAE/g honey)	(%) Inhibition DPPH
A1	14.199±0.26 <sup>no</sup> rs	104.180±2.94 <sup>rs</sup> tu	36.245±0.82 <sup>rs</sup>
A2	17.030±0.38 <sup>r</sup>	128.673±1.99 <sup>r</sup>	48.927±0.56 <sup>klmn</sup>
A3	16.194±1.49 <sup>rs</sup> y	104.866±1.90 <sup>rs</sup> tu	51.366±1.69 <sup>mno</sup> pr
A4	16.726±0.29 <sup>rs</sup> y	107.386±5.21 <sup>rs</sup> uv	54.445±0.18 <sup>rs</sup> tu
A5	13.548±0.35 <sup>klmno</sup> pr	90.680±4.80 <sup>defghijkl</sup>	52.196±0.83 <sup>op</sup> rst
A6	13.734±0.19 <sup>klmno</sup> pr	99.376±1.77 <sup>mno</sup> prst	54.592±1.12 <sup>rs</sup> tu
A7	13.776±0.52 <sup>klmno</sup> pr	105.553±0.80 <sup>rs</sup> tu	50.163±0.07 <sup>klmno</sup>
A8	12.627±0.72 <sup>ghijklmn</sup>	98.615±3.12 <sup>lmno</sup> rs	48.295±0.28 <sup>klm</sup>
A9	11.511±0.70 <sup>de</sup> fghi	104.323±2.52 <sup>rs</sup> tu	49.264±2.09 <sup>klmno</sup>
A10	15.670±0.37 <sup>stuv</sup> y	118.676±1.21 <sup>r</sup>	51.700±1.39 <sup>no</sup> rs
A11	15.771±0.46 <sup>stuv</sup> y	114.256±1.12 <sup>rs</sup> tu	58.109±0.70 <sup>r</sup>
A12	10.615±0.55 <sup>cdef</sup>	115.243±0.22 <sup>r</sup>	54.630±1.23 <sup>rs</sup> tu
A13	10.860±0.99 <sup>de</sup> fg	99.606±2.89 <sup>mno</sup> prst	65.437±0.44 <sup>r</sup>
A14	19.412±0.01 <sup>r</sup>	96.860±0.73 <sup>klmno</sup> pr	37.241±0.03 <sup>s</sup>
A15	9.102±0.09 <sup>bc</sup>	101.131±3.49 <sup>mno</sup> prst	50.489±1.98 <sup>mno</sup>
A16	11.334±0.69 <sup>de</sup> fghi	106.543±1.60 <sup>rs</sup> tu	55.060±0.62 <sup>rs</sup>
A17	11.735±0.00 <sup>de</sup> fghij	99.604±0.92 <sup>mno</sup> prst	35.842±0.06 <sup>rs</sup>
A18	11.731±0.55 <sup>de</sup> fghij	106.930±1.65 <sup>rs</sup> tu	51.846±0.20 <sup>no</sup> rs
A19	11.486±0.28 <sup>de</sup> fghi	103.186±0.35 <sup>op</sup> rst	52.035±0.63 <sup>no</sup> prst
A20	8.409±0.32 <sup>ab</sup>	93.808±1.26 <sup>ghijklmn</sup>	36.046±0.28 <sup>rs</sup>
A21	10.505±0.16 <sup>cdef</sup>	96.250±4.36 <sup>klmno</sup> pr	46.824±0.54 <sup>ij</sup>
A22	13.329±0.58 <sup>klmno</sup> p	93.122±2.42 <sup>ghijklmn</sup>	47.547±0.10 <sup>kl</sup>
A23	16.084±0.23 <sup>stuv</sup> y	87.704±0.57 <sup>bcde</sup> fg	56.880±0.75 <sup>rs</sup> tu
A24	14.681±1.53 <sup>rs</sup> tu	108.073±0.95 <sup>rs</sup> tu	46.302±0.43 <sup>hij</sup>
A25	12.276±0.00 <sup>de</sup> fghijklm	96.555±1.83 <sup>klmno</sup> pr	46.425±2.21 <sup>hij</sup>
A26	11.452±0.40 <sup>de</sup> fghi	92.435±1.17 <sup>de</sup> fghijklm	43.926±0.51 <sup>hi</sup>
A27	14.165±0.62 <sup>no</sup> rs	84.729±1.94 <sup>bcde</sup>	47.193±1.52 <sup>jk</sup>
A28	23.201±0.79 <sup>r</sup>	86.636±1.27 <sup>bcde</sup> fg	36.515±0.27 <sup>s</sup>
A29	11.782±0.76 <sup>de</sup> fghij	91.520±2.84 <sup>de</sup> fghijklm	35.960±0.38 <sup>rs</sup>
A30	19.735±0.30 <sup>r</sup>	93.656±3.07 <sup>ghijklmn</sup>	53.796±1.45 <sup>rs</sup> tu
A31	12.095±0.44 <sup>de</sup> fghijkl	90.222±0.95 <sup>de</sup> fghijk	43.590±1.26 <sup>r</sup>
A32	19.315±1.00 <sup>r</sup>	80.838±1.86 <sup>ab</sup>	52.363±0.65 <sup>op</sup> rst
A33	10.336±1.29 <sup>cde</sup>	93.198±2.05 <sup>de</sup> fghijklmn	31.618±2.07 <sup>cde</sup>
A34	11.461±0.63 <sup>de</sup> fghi	95.868±3.91 <sup>hijklmno</sup>	29.918±0.57 <sup>r</sup>
A35	11.452±0.19 <sup>de</sup> fghi	91.290±1.99 <sup>de</sup> fghijklm	34.179±2.48 <sup>ef</sup>
A36	19.110±0.51 <sup>r</sup>	109.140±2.86 <sup>rs</sup> tu	36.955±2.56 <sup>s</sup>
A37	6.896±0.19 <sup>a</sup>	85.263±0.22 <sup>abcde</sup> fg	25.057±1.01 <sup>a</sup>
A38	12.678±1.09 <sup>ghijklmn</sup>	81.372±4.04 <sup>abc</sup>	30.914±0.70 <sup>cd</sup>
A39	11.613±0.53 <sup>de</sup> fghij	84.423±4.85 <sup>bcde</sup>	34.431±0.51 <sup>ef</sup>
A40	11.844±0.04 <sup>de</sup> fghijk	78.549±1.55 <sup>a</sup>	36.995±0.58 <sup>s</sup>
A41	8.397±0.06 <sup>ab</sup>	87.933±1.52 <sup>bcde</sup> fg	30.423±0.88 <sup>cd</sup>
A42	11.048±0.05 <sup>de</sup> fghi	94.724±6.45 <sup>ghijklmn</sup>	33.194±1.50 <sup>def</sup>
A43	10.376±0.00 <sup>cde</sup>	96.095±4.24 <sup>ijklmno</sup>	24.647±1.01 <sup>a</sup>
A44	11.845±0.23 <sup>de</sup> fghijk	89.307±4.54 <sup>de</sup> fghijk	28.578±0.37 <sup>bc</sup>
A45	14.458±0.00 <sup>op</sup> qrst	88.773±1.34 <sup>bcde</sup> fg	26.393±0.64 <sup>ab</sup>
A46	12.790±0.01 <sup>ijklmno</sup>	83.508±2.84 <sup>abcd</sup>	47.265±0.01 <sup>jk</sup>
A47	10.121±0.00 <sup>cd</sup>	83.432±1.42 <sup>abcd</sup>	29.233±0.01 <sup>c</sup>
A48	13.911±0.12 <sup>mno</sup> pr	87.781±1.04 <sup>bcde</sup> fg	48.565±0.01 <sup>klm</sup>
A49	15.239±0.08 <sup>stuv</sup>	90.527±1.99 <sup>de</sup> fghijkl	50.688±0.02 <sup>mno</sup> p
A50	10.915±0.01 <sup>de</sup> fghi	78.091±1.68 <sup>a</sup>	36.866±0.00 <sup>s</sup>

Means in the same row with different letters are significantly different according to Tukey's test (p<0.05), The values represent average±standard deviation, (n = 3)

2010; Bertonecclj *et al.*, 2007, Ouchemoukh *et al.*, 2007; Kucuk *et al.*, 2007, Akbulut *et al.*, 2009). For example, Saxena *et al.*, 2010) reported that the Indian honeys 47-98 mg GAE/ 100 g, Al-Mamary *et al.*, 2002 reported that the total phenols of Yemeni honeys ranged from 56.32-246.21 mg/100 g, Al *et al.* (2009) Romanian honeydew honeys 23.0-125.0 mg GAE/100 g. Gheldof *et al.* (2002) reported that acacia honeys had lower phenolic content (4.6 mg GAE/100 g) than sunflower honeys. Al-Mamary *et al.* (2002) indicated that the

determination of total phenolic content of honey is a good parameter for the assessment of its quality and possible therapeutic potential.

**Antioxidant activity of sunflower honeys:** Phosphomolybdenum assay was introduced for the measurement of the antioxidant activity of the sunflower honeys. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green Mo (V) complexes

with a maximal absorption at 695 nm (Prieto *et al.*, 1999). The antioxidant power of the honey samples varied from  $78.091 \pm 1.68$ - $128.673 \pm 1.99$  mg AAE/g honey (Table 3). In this study, antioxidant capacities were detected to be lowest in A40 and A50 samples and highest in A2. The statistical differences among the antioxidant activity of the sunflower honeys were important ( $p < 0.05$ ).

Bertoncelj *et al.* (2007), measured antioxidant activity in Slovenian honeys, found the lowest antioxidant activity in acacia and lime honeys and the highest in dark-colored honeys, such as those from spruce trees. Kucuk *et al.* (2007) stated that sunflower honeys exhibited the highest antioxidant power in tested honey samples. Lachman *et al.* (2010), who measured antioxidant activity in Czech honeys, highest values of antioxidant activity showed unambiguously honeydew honeys and conversely the lowest values demonstrated floral honeys. In addition, Buratti *et al.* (2007) reported that the variation in the antioxidant power among unifloral honeys with different geographical origin can be due to climate and environmental factors such as humidity, temperature and soil composition.

**Antiradical activity of sunflower honeys:** The free radical scavenging activity of the methanolic extracts of the tested sunflower honeys were determined through the DPPH method and the results are presented in Table 3. The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical which resulted in the bleaching of the purple color exhibited by the stable DPPH radical, was monitored at an absorbance of 517 nm. The free radical scavenging activity of sunflower honey samples varied from  $24.647 \pm 1.01\%$  to  $65.437 \pm 0.44\%$ . In this study while the lowest antiradical activity was determined in A43 honey, it was highest in A13 honey. The statistical differences among the total antiradical activity of the sunflower honeys were important ( $p < 0.05$ ).

Saxena *et al.* (2010) reported that free radical scavenging activity in Indian honeys 44-71%, Akbulut *et al.* (2009) reported that free radical scavenging activity of pine honey were 25.65-50.78%. Our findings were approximately similar with these results. In addition Kucuk *et al.* (2007) reported that chestnut honeys exhibited the highest free radical scavenging activity when compared with two other honeys.

Bivariate correlations were analyzed by Pearson's test using SPSS 17.0 on Windows. It was observed that there is no correlation between the antioxidant activities and total phenolic contents of the sunflower honeys (Pearson's correlation coefficient = 0.173). However, Regression analysis between antiradical capacity and total phenolic content showed a linear correlation

( $p < 0.05$ ). Results indicated that antiradical activity was related to the phenolic contents of the sunflower honeys. In previous studies, it has been shown a strong relationship between antiradical capacity and the total phenolic content of honey (Baltrusaityte *et al.*, 2007; Buratti *et al.*, 2007). For example, Akbulut *et al.* (2009) reported that the strong correlation was found between the phenolic content and antiradical activity of pine honeys ( $r = 0.89$ ), (Beretta *et al.*, 2005) found that correlation ( $r = 0.918$ ).

## CONCLUSION

As a result, the majority of the examined honey samples were found to be in compliance with TSE 3036, the Codex and EU honey standards. However, the moisture and acidity value of some samples were found to be higher than the standards. This situation could be attributed to the incorrect processes applied by honey producers in storage and preservation.

It was demonstrated that the 50 sunflower honeys obtained from the Thrace region of Turkey have stronger antioxidant and antiradical activities but lower phenolic content. The antioxidant and antiradical activity of sunflower honeys may contribute to the treatment of different illnesses. At the same time, the prediction of phenolic contents, antiradical and antioxidant powers of honeys may be an attractive source of nutraceuticals and medicinal ingredients.

## ACKNOWLEDGMENTS

We would like to thank Erciyes University's Scientific Research Project Unit (Project No: FBD-09-966) for its financial support of this work. This paper includes data extracted from the doctoral thesis of the first author.

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