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Total Phenolic Acid and Total Flavonoid Content of Turkish Pine Honeydew Honey

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Summary

From September to November during the years between 2004 and 2006, 78 honey samples were collected from ten areas of the Muğla city in western Turkey, Merkez, Milas, Ortaca, Köyceğiz, Marmaris, Fethiye, Yatağan, Bodrum, Ula and Datça where pine honey beekeeping is practiced extensively. Using light microscopy the Number of Honeydew Elements (NHE) and the Number of Total Pollen (NTP) were determined and samples with an NHE / NTP ratio above 4.5 were accepted as high density, superior quality pine honeydew honey. Of the 78, honeys collected, 50 were found to be pine honeydew honeys and were analysed for total phenolic and total favonoids. The mean total phenolic acids of the 50 pine honeys were found to be 155.55 mg GAE/kg, with a minimum of 35.36 mg GAE/kg, and a maximum of 365.94, while the mean total flavonoid content was found to be 22.80 mg QE/kg, with a minimum of 4.80 mg QE/kg, and a maximum of 54.78 mg QE/kg.

Keywords: Pine honey, Total flavonoid content, Total phenolic acid, Muğla.

Introduction

Honeydew honey is prepared from secretions of living parts of plants or excretions of plant-sucking insects on the living part of plants (Sanz *et al.*, 2005). Honeydew is the origin of pine honey, it refers to honey produced by honeybees collecting nectars which are exuded from other insects such as aphids or scale insects (Zander and Koch, 1994). Insects take essential nutrition from concentrated sugar solution in the phloem and exude the remains. Honeybees collect these exudates and turn them into honey in the hive. This honey is called honeydew honey (Zander and Koch, 1994).

In Turkey Marchalina hellenica (syn. Monophlebus hellenicus) (Coccidea: Homoptera), which lives on Pinus brutia, is the main source of honeydew. This insect is found only in Turkey and Greece (Santas, 1979) mainly in Southern Marmara, the Aegean and West Mediterranean regions of Turkey (Gurkan, 1989). In Turkey, about 30% of honey is produced in the region of Mugla which is characterized by having nearly 60 000 hectare of Pinus

brutia forest, hence it is a important city for the production of pine honey produced from honey dew of *Marchalina hellenica* (Sahin,2000). Besides *Pinus brutia, Eucalyptus camaldulensis* Dehnh., *Liquidambar orientalis* Miller, *Pinus pinea* L., *Ceratonia siliqua* L., *Thymus* L. and other floral plants are the important honey origins in Muğla (Şahin, 2000).

Antioxidant activity is the ability and potential of honey in reducing oxidative reactions within food systems. Notably, these oxidative reactions can cause deleterious reactions in food products (e.g. lipid oxidation in meat, and enzymic browning in fruits and vegetables) and adverse human health effects, such as chronic diseases and cancers (Gheldof and Engeseth, 2002). The antioxidants that naturally occur in honey contribute to its antioxidant capacity (Nicholls and Miraglio, 2003). These compounds are polyphenols (flavonoids and phenolic acids), some enzymes (e.g. glucose oxidase, catalase), ascorbic acid, carotenoid-like substances, organic acids, proteins and amino acids (Gheldof *et al.*, 2002). Honey contains at least four broad groups of components with antioxidant activity,

which are polyphenols or phenolic compounds (flavonoids and phenolic acids), enzymes (e.g. glucose oxidase, catalase), enzymes, ascorbic acid, and peptides (Gheldof *et al.*, 2002; Nicholls and Miraglio, 2003). In our study we have determined the polyphenols in pine honey samples.

Plant polyphenols are primary natural antioxidants. They have many beneficial functions such as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers (Kühnau, 1976; Shahidi et al., 1992; Duthie et al., 2000). Flavonoids and simple phenolic derivatives such as phenolic acids are among the most common classes representing the majority of plant polyphenols (Bravo, 1998). Both of them have been considered as important components generating antioxidant properties in honey.

Flavonoids are low molecular weight phenolic compounds based on the flavan nucleus (Coultate, 1996), and specified by the presence of a C_6 - C_3 - C_6 carbon skeleton (Peterson and Dwyer, 1998; Skibola and Smith, 2000). They can be classified into 13 different major classes depending on their basic structures. Flavones, flavonols, and glycosides are among the most common classes (Bravo, 1998).

In monofloral honeys, flavonoids are often found as major components, which are up to 42% of total phenolics (Sabatier *et al.,* 1992). However, flavonoids profiles in honeys generally vary widely and they are mainly different according to floral and geographical origins (Soler *et al.,* 1995).

Phenolic acids (aromatic carbonic acids) are a subclass of the most numerous and ubiquitous groups of secondary plant metabolites referred to as "phenolics" (Anklam, 1998; Bravo, 1998; Robbins, 2003).

Their chemical structure is simple C_6 - C_1 . But they are associated with a distinct group of organic acids having three distinguishing constitutive carbon frameworks, which are the hydroxycinnamic, hydroxybenzoic structure, and in many cases also an aldehyde analogue. Variations among their structures are due to differences in the numbers and position of the hydroxyl groups on the aromatic ring (Robbins, 2003).

Honey phenolics may be classified into three groups. Two of them, which are benzoic acids and their esters, cinnamic acids and their esters, are phenolic acids (Sabatier et al., 1992). It has been reported that benzoic acids and their esters are the most common phenolic acids found in honey (Sabatier et al., 1992).

Within honey, phenolic acids are not only present but may also define certain types of honey. Honeydew, chesnut, and forest blossom honey can be differentiated by a careful evaluation of the distribution pattern of phenolic acids (Anklam, 1998).

Materials and methods

Collection of Honey Samples

From September to November in the years between 2004 and 2006 a total of 78 honey samples were collected from ten areas of the Muğla city in western Turkey; Merkez (4), Milas (8), Ortaca (3), Köyceğiz (7), Marmaris (12), Fethiye (13), Yatağan (11), Bodrum (1), Ula (9) and Datça (10) where pine honey beekeeping is practiced extensively. In this study, suitable apiaries were chosen from villages. Muğla has 12851 km² acreage. It was important to assure the presence of enough distance between villages and the presence of enough variation in vegetation which characterized Muğla.

Microscopic Analysis of Honeys

All honey samples were examined under the light microscope (Nikon Eclipse E400), and methods described by Moar (1985) and Sorkun (2008) were followed. The Number of Honeydew Element (NHE) and the Number of Total Pollen (NTP) in these samples were investigated and the quality of the samples was determined on the basis of the NHE/NTP ratio. Each honey sample having a NHE/NTP ratio higher than 4.5 was accepted as a high density superior quality pine honeydew honey (Table 1).

Table 1. Classification of honey samples by NHE/NTP

NHE/NTP	Identification	Honey type
0-1,5	Low density	Floral honey
1,5-3,0	Medium density	Pine + floral honey
3,0-4,5	Dense	Pine honey
> 4,5	Very dense	Superior quality pine honey

Following microscopic examinations, 50 honey samples were determined as pine honey samples and found to be appropriate for Total Phenolic and Total Flavonoid analysis.

Total Phenolic Acid Analysis

The total phenolic content of honey samples was determined by the Folin-Ciocalteu method (Hoerudin, 2004).

a) Preparation of Standards

To calculate the total phenolic content, which was expressed as gallic acid equivalent (GAE), a standard curve of known concentrations of gallic acid was constructed. The standard solutions were prepared in five different concentrations, which were 0, 25, 50, 75, and 100 mg/L. Firstly, a stock gallic acid solution was made up by dissolving 25 mg of gallic acid in 100 mL of 70% methanol. Then, to make up

the standard working solutions, 0, 1, 2, 3, and 4 mL aliquots of a stock gallic acid solution (250 mg/L) was separately pipetted into a 10 mL volumetric flask and subsequently diluted to volume with 70% methanol.

To generate the standard curve, 1 mL of each of the standard solutions was pipetted into a seperate test tube. Then 5 mL of a 10% aqueous dilution of Folin-Ciocalteu reagent was added and mixed well using a vortex mixer for about 1 min. After 3-8 minutes, 4 mL of a 75 g/L anhydrous Na_2CO3 solution was added. The mixture was mixed thoroughly for another 1 min and incubated in water bath at 45°C for 15 min.

b) Preparation of Samples

Five grams from each honey sample were dissolved in 50 mL of 70% methanol. One mL from each methanolic honey solution was transfered to a test tube. It was then treated and measured by the procedure used for constructing the standard curve.

A sample blank was prepared for each honey sample by pipetting 20 mL of the honey extract into a 100 mL glass beaker and adjusting its pH value to 3.5 by adding of 20% acetic acid. After the addition of 1.6 g of polyvinylpolypyrrolidone (PVPP), the honey extract was shaken gently using a mechanical shaker (IKA Laboratechnik KS130 Basic) at 320 rpm for 20 min. The PVPP mixture was filtered through Whatman filter paper No.1 and 1 mL of filtrate was taken and tested as the above procedures. After cooling, the absorbance of standards, sample solutions and references were read in a UV/Visible Spectrophotometer at 765 nm against a zero absorbance blank. The following formula was used to calculate the final total phenolic acid content:

Total phenolic content (mg GAE/kg honey)=

GAE (mg/L) x total volume of methanol extract (mL) x

10⁻³(L/mL) x dilution factor

Sample weight (g) x 10⁻³(kg/g)

Total Flavonoid Analysis

The total flavonoid content of honeys was estimated by aluminium chloride (AlCl₃) colorimetric method (Hoerudin, 2004).

a) Preparation of Standards

To quantify the total flavonoid content, quercetin was used as the reference, which was expressed as quercetin equivalent (QE). A standard curve of known concentrations of quercetin was generated by preparing and testing five concentrations of quercetin standard solution, which were 0, 25, 50, 75, and 100 mg/L. A stock quercetin solution was prepared by dissolving 25 mg of quercetin in 100 mL of 80% ethanol. Then, the standard working solutions were made up

by pipetting 0, 1, 2, 3, and 4 mL aliquots of the stock solution (250 mg/L) into 10 mL-volumetric flasks and adjusting the volume with 80% ethanol. By using test tubes, 1 mL of each standard solution was reacted with 3 mL of 95% ethanol, 0.2 mL of a 10% aqueous dilution of AlCl $_3$ reagent, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. The mixture was mixed thoroughly by vortex mixer for about 30 s and allowed to stand at room temperature for 30 min. Absorbance readings were taken by a UV/Visible Spectrophotometer at 415 nm.

b) Preparation of Samples

Five gram of each honey sample were dissolved in 25 mL of 80% methanol. One ml of each honey solution was transferred to a test tube. Subsequently, similar procedures used for constructing the standard curve were applied to assay 1 mL of each of standard solutions. A sample blank was similarly prepared for each honey sample, but the same amount of 10% AlCl₃ solution (0.2 mL) was replaced by distilled water. Absorbance readings were taken by a UV/ Visible Spectrophotometer at 415 nm. The following formula was used to calculate the final total phenolic acid content:

Total flavonoid content (mg QE/kg honey)=

QE (mg/L) x total volume of ethanol extract

(mL x 10-3(L/mL) x dilution factor

Sample weight (g) x 10-3(kg/g)

Results

Following the microscopic analysis of 78 honey samples (Table 2), 50 honey samples were determined as high density superior quality pine honey and selected for total phenolic acid and flavonoid analysis.

Results in Table 3 shows that the mean total phenolic acids of the 50 examined pine honey samples was 155.55 mg GAE/kg. The minimum detected amount was 35.36 mg GAE/kg, while the maximum one was 365.94 mg GAE/kg. Regarding the total flavonoid content, it was determined at a mean of 22.80 mg QE/kg, with a minimum of 4.80 mg QE/kg and a maximum of 54.78 mg QE/kg.

Table 2. Outcomes of NHE/NTP analysis of honey samples.

Sample	District-village	NHE	NTP	NHE/NTP	Identification	
1	Yatağan-Şerefköy	2528	93919	0.02	Low density floral honey	
2	Yatağan-Şerefköy	2752	6882	0.4	Low density floral honey	
3	Yatağan-Hacıveliler	3836	16526	0.23	Low density floral honey	
4	Yatağan-Hacıveliler	15176	58178	0.26	Low density floral honey	
5	Yatağan-Turgutlar	2068	50685	0.04	Low density floral honey	
6	Yatağan-Turgutlar	6318	225947	0.03	Low density floral honey	
7	Ula-Kıyra	31412	17816	1.76	Medium density pine + floral honey	
8	Ula-Kıyra	58946	16806	3.50	Dense pine honey	
9	Datça-Palamutbükü	26684	15121	1.76	Medium density pine + floral honey	
10	Datça-Palamutbükü	12656	128411	0.09	Low density floral honey	
11	Datça-Sındı	59408	9995	5.94	High density superior quality pine honey	
12	Datça-Sındı	55770	10940	5.09	High density superior quality pine honey	
13	Datça-Hızırşah	43326	8795	4.92	High density superior quality pine honey	
14	Datça-Hızırşah	31418	13175	2.38	Medium density pine + floral honey	
15	Marmaris-Çamlı	166366	9222	18.04	High density superior quality pine honey	
16	Marmaris-Çamlı	189892	11758	16.15	High density superior quality pine honey	
17	Marmaris-Çamlı	127180	39606	3.21	Dense pine honey	
18	Marmaris-Çamlı	79812	38462	2.07	Medium density pine + floral honey	
19	Marmaris-Çetibeli	66284	12736	5.2	High density superior quality pine honey	
20	Marmaris-Çetibeli	58290	1247272	0.04	Low density floral honey	
21	Marmaris-Turgut	118880	26391	4.5	High density superior quality pine honey	
22	Marmaris-Turgut	87082	20734	4.2	Dense pine honey	
23	Marmaris-Orhaniye	168200	28623	5.87	High density superior quality pine honey	
24	Marmaris-Orhaniye	128168	12370	10.36	High density superior quality pine honey	
25	Ortaca-Karadonlar	55944	1832	30.5	High density superior quality pine honey	
26	Fethiye-Çatak	3754	6608	0.56	Low density floral honey	
27	Fethiye-Çatak	8946	6390	1.4	Low density floral honey	
28	Fethiye-Dere	498544	4703	106	High density superior quality pine honey	
29	reality c Dere		-1700	1	mgir actionly superior quanty pine noney	
29	Fethiye-Dere	146542	8449	17.3	High density superior quality pine honey	
30				17.3 0.95		
	Fethiye-Dere	146542	8449 42730 12069	0.95 2.02	High density superior quality pine honey Low density floral honey Medium density pine + floral honey	
30	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç	146542 40980 24454 3622	8449 42730 12069 28428	0.95 2.02 0.12	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey	
30 31	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç	146542 40980 24454	8449 42730 12069	0.95 2.02	High density superior quality pine honey Low density floral honey Medium density pine + floral honey	
30 31 32	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç	146542 40980 24454 3622	8449 42730 12069 28428	0.95 2.02 0.12	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey	
30 31 32 33	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç	146542 40980 24454 3622 13682	8449 42730 12069 28428 23183	0.95 2.02 0.12 0.59	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey	
30 31 32 33 34	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar	146542 40980 24454 3622 13682 115481	8449 42730 12069 28428 23183 1419	0.95 2.02 0.12 0.59 81	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey	
30 31 32 33 34 35	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar	146542 40980 24454 3622 13682 115481 65323	8449 42730 12069 28428 23183 1419	0.95 2.02 0.12 0.59 81	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey High density superior quality pine honey	
30 31 32 33 34 35 36	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar Centre-Dağpınar	146542 40980 24454 3622 13682 115481 65323 138817	8449 42730 12069 28428 23183 1419 871 6271	0.95 2.02 0.12 0.59 81 75 22.13	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey High density superior quality pine honey High density superior quality pine honey	
30 31 32 33 34 35 36 37	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar Centre-Dağpınar Centre-Kıran	146542 40980 24454 3622 13682 115481 65323 138817 136963	8449 42730 12069 28428 23183 1419 871 6271 13318	0.95 2.02 0.12 0.59 81 75 22.13	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey	
30 31 32 33 34 35 36 37	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar Centre-Dağpınar Centre-Kıran Ula-Akyaka	146542 40980 24454 3622 13682 115481 65323 138817 136963 70007	8449 42730 12069 28428 23183 1419 871 6271 13318 14366	0.95 2.02 0.12 0.59 81 75 22.13 10.28	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey	
30 31 32 33 34 35 36 37 38 39	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar Centre-Dağpınar Centre-Kıran Ula-Akyaka Ula-Akyaka	146542 40980 24454 3622 13682 115481 65323 138817 136963 70007 184709	8449 42730 12069 28428 23183 1419 871 6271 13318 14366 3990	0.95 2.02 0.12 0.59 81 75 22.13 10.28 4.87 46.3	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey	
30 31 32 33 34 35 36 37 38 39 40	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar Centre-Dağpınar Centre-Kıran Ula-Akyaka Ula-Akçapınar	146542 40980 24454 3622 13682 115481 65323 138817 136963 70007 184709 137840	8449 42730 12069 28428 23183 1419 871 6271 13318 14366 3990 11933	0.95 2.02 0.12 0.59 81 75 22.13 10.28 4.87 46.3 11.5	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey	
30 31 32 33 34 35 36 37 38 39 40	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar Centre-Dağpınar Centre-Kıran Ula-Akyaka Ula-Akçapınar	146542 40980 24454 3622 13682 115481 65323 138817 136963 70007 184709 137840 102744	8449 42730 12069 28428 23183 1419 871 6271 13318 14366 3990 11933 1605	0.95 2.02 0.12 0.59 81 75 22.13 10.28 4.87 46.3 11.5	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey	
30 31 32 33 34 35 36 37 38 39 40 41	Fethiye-Dere Fethiye-Bağlıağaç Fethiye-Bağlıağaç Fethiye-Kabaağaç Fethiye-Kabaağaç Centre-Dağpınar Centre-Dağpınar Centre-Kıran Ula-Akyaka Ula-Akçapınar Ula-Akçapınar Datça-Sındı	146542 40980 24454 3622 13682 115481 65323 138817 136963 70007 184709 137840 102744 65497	8449 42730 12069 28428 23183 1419 871 6271 13318 14366 3990 11933 1605 1950	0.95 2.02 0.12 0.59 81 75 22.13 10.28 4.87 46.3 11.5 64 33.5	High density superior quality pine honey Low density floral honey Medium density pine + floral honey Low density floral honey Low density floral honey High density superior quality pine honey	

Table 2 (cont.) Outcomes of NHE/NTP analysis of honey samples

No	District-village	NHE	NTP	NHE/NTP	Identification	
46	Fethiye-Ören	22717	7809	2.9	Medium density pine + floral honey	
47	Fethiye-Ören	89884	10451	8.6	High density superior quality pine honey	
48	Fethiye-Ören	116258	6107	19	High density superior quality pine honey	
49	Fethiye-Ören	116040	5889	19	High density superior quality pine honey	
50	Ortaca-Gökbel	123341	12888	9.5	High density superior quality pine honey	
51	Ortaca-Gökbel	121619	5320	22.8	High density superior quality pine hone	
52	Köyceğiz-Döğüşbelen	95401	113494	0.84	Low density floral honey	
53	Köyceğiz-Döğüşbelen	118916	19277	6.16	High density superior quality pine honey	
54	Köyceğiz-Döğüşbelen	82534	25893	3.1	Dense pine honey	
55	Köyceğiz-Döğüşbelen	70742	25647	2.7	Medium density pine + floral honey	
56	Milas-Bozbük	75542	16802	4.5	High density superior quality pine honey	
57	Milas-Bozbük	142085	11854	11.9	High density superior quality pine honey	
58	Milas-Pınar	213810	2388	89.53	High density superior quality pine honey	
59	Milas-Pinar	67215	731	92	High density superior quality pine honey	
60	Milas-Kayabükü	110731	7231	15.3	High density superior quality pine honey	
61	Milas-Kayabükü	135453	5495	24.6	High density superior quality pine honey	
62	Yatağan-Bencik	152800	3200	47	High density superior quality pine honey	
63	Yatağan-Bencik	223367	3400	77	High density superior quality pine honey	
64	Yatağan-Bencik	121500	4507	26.9	High density superior quality pine honey	
65	Yatağan-Bencik	143606	2508	57.2	High density superior quality pine honey	
66	Milas-Çukurköy	214864	9241	23.2	High density superior quality pine honey	
67	Milas-Çukurköy	167851	5860	28.6	High density superior quality pine honey	
68	Ula-Karaböğütlen	51719	7499	6.9	High density superior quality pine honey	
69	Ula-Karaböğütlen	46747	7069	6.6	High density superior quality pine honey	
70	Köyceğiz-Toparlar	61548	4645	13.25	High density superior quality pine honey	
71	Köyceğiz-Toparlar	26977	15224	1.7	Medium density pine + floral honey	
72	Datça-Mesudiye	77949	5124	15.2	High density superior quality pine honey	
73	Datça-Mesudiye	92446	9507	9.7	High density superior quality pine honey	
74	Yatağan-Bağyaka	266578	232395	1.14	Low density floral honey	
75	Ula-Kızılyaka	182940	224242	0.81	Low density floral honey	
76	Bodrum-Gölköy	316190	40926	7.72	High density superior quality pine honey	
77	Köyceğiz-Ekincik	390004	32987	11.82	High density superior quality pine honey	
78	Fethiye-Kabaağaç	192481	23292	8.26	High density superior quality pine honey	

Samples in bold font were chosen for the Total Phenolic acid and Total Flavonoid analysis.

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Table 3. Total Phenolic Acid and Flavonoid levels in Pine Honey Samples*.

Sample	Mean total phenolic acid content	Mean total flavonoid content	
11	235.72±0.28	15.27±2.55	
12	189.57±0	12.03±0.60	
13	220.72±0.27	18.50±0	
15	365.22±0.90	28.70±2.48	
16	219.84±0.28	10.84±0	
19	339.91±0.93	34.15±0.54	
21	365.94±0	15.05±6.28	
23	129.06±0	30.50±0.59	
24	50.52±0	37.29±0.91	
25	51.52±0	26.59±8.26	
28	128.15±0	22.37±6.12	
29	93.05±1.30	18.94±2.97	
34	131.19±0	27.51±2.26	
35	152.94±0.70	23.51±4.08	
36	97.32±0	26.28±1.49	
37	101.17±0.95	26.16±4.52	
38	84.74±2.72	28.74±0.47	
39	112.46±1.87	19.16±2.17	
40	223.47±0.30	29.48±2.50	
41	211.99±0.30	24.22±4.19	
42	179.50±1.51	26.62±1.92	
43	140.94±0.91	21.80±1.37	
44	146.74±2.65	30.31±7.31	
45	105.10±4.01	24.12±5.27	
47	61.90±0.77	11.87±1.18	
48	122.66±2.46	22.25±1.26	
49	116.42±3.48	21.31±0.84	

Sample	Mean total phenolic acid content	Mean total flavonoid content
50	196.10±1.61	31.14±1.45
51	140.99±3.51	21.98±4.00
53	158.92±0.61	24.03±1.30
56	110.19±3.16	18.73±0.48
57	161.70±3.56	9.99±1.25
58	186.41±3.30	4.80±1.60
59	163.51±0	26.36±2.71
60	143.50±4.74	14.86±0.37
61	156.58±2.19	16.94±8.58
62	143.04±0.92	18.63±0.15
63	184.32±1.02	18.02±1.36
64	152.99±0.02	27.68±4.99
65	160.12±2.45	16.46±4.04
66	102.23±8.82	23.33±1.49
67	96.60±12.32	27.23±0.03
68	101.47±1.52	25.15±0.49
69	95.07±8.96	18.30±4.07
70	35.36±0.31	11.39±0
72	122.98±2.92	11.61±0.68
73	171.07±5.78	32.71±2.49
76	211.54±0.69	25.35±4.03
77	158.36±0.96	26.80±1.10
78	246.71±6.12	54.78±4.06
Mean	155.55±2.04	22.80±2.45
Minimum result	35.36±0.31	4.80±1.60
Maximum result	365.94±0.90	54.78±4.06

Discussion

In this study, total phenolic content (mg of GAE/kg of honey) varied from 35.36 to 365,94 mg GAE/kg with a mean of 155,55 using a standard curve of gallic acid ($\rm R^2=0.9988$). It has been reported that total phenolic acid contents of Australian unifloral honeys ranged from 14 to 195.96 mg GAE/kg (Hoerudin, 2004). Meda *et al.*, (2005), determined that total phenolic content (mg of GAE/100 g of honey) varied from 32.59 to 114.75 mg with a mean of 74.38 \pm 20.54 mg. They found that honeydew honeys had higher levels of phenolic compounds than the other honey samples which they investigated; for two honeydew honey samples they found 113.05 mg GAE/100 g and 114.75 mg GAE/100 g respectively.

Using the standard curve generated by quercetin ($R^2 = 0.9999$), the total flavonoid content of honey samples (mg of QE/kg of honey) was found in this study to vary from 4.80 to 54.78 mg with a mean value of 22.80 mg. It has been reported that in Australian unifloral honeys total flavonoid content ranged from 8.81

to 45.04 mg QE/kg (Hoerudin, 2004). Meda *et al.*, (2005) determined lower ranges of total flavonoid content (mg of QE/100 g of honey) that varied from 0.17 to 8.35 mg and with a mean of 2.57 \pm 2.09 mg. Moreover, low correlation was determined (R = - 0.38) between total phenolic and total flavonoid content of the samples examined in this study. This is consistent with results by Meda *et al.*, (2005) who also found low correlation (R = 0.11) between total phenolic and total flavonoid content and with those by Hoerudin (2004), who determined correlations in Jarrah, Yapunya and Leatherwood honeys (0.96, -0.40 and 0.27 respectively).

Flavanoids and phenolic acids are examples of antioxidants, which are important ingredients of many foods, and keenly sought in many 'health foods'. They are thought to help protect against diseases like cancer, cardiovascular disorders, neurodegenerative diseases and ageing by mopping up potentially damaging free radicals that are released in the body. Honey is a source of antioxidants and honeydew honey has been shown to contain high total polyphenol content (Vela, de Lorenzo & Pérez, 2007).

^{*} Mean \pm S.D (n = 3)

Conclusion

This study showed that the 50 samples of Muğla Turkish pine honey contained phenolic compounds and flavonoids in variable quantities. The correlation between Total Phenolic Acid and Total Flavanoid seems to depend on the composition of honey and further chemical investigations are needed to evaluate Turkish pine honey.

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Total Phenolic Acid and Total Flavonoid Content of Turkish Pine Honeydew Honey

Author(s)
Aslı Özkök, Bruce D'arcy, Kadriye Sorkun

From September to November during the years between 2004 and 2006, 78 honey samples were collected from ten areas of the Muğla city in western Turkey, Merkez, Milas, Ortaca, Köyceğiz, Marmaris, Fethiye, Yatağan, Bodrum, Ula and Datça where pine honey beekeeping is practiced extensively. Using light microscopy the Number of Honeydew Elements (NHE) and the Number of Total Pollen (NTP) were determined and samples with an NHE / NTP ratio above 4.5 were accepted as high density, superior quality pine honeydew honey. Of the 78, honeys collected, 50 were found to be pine honeydew honeys and were analysed for total phenolic and total favonoids. The mean total phenolic acids of the 50 pine honeys were found to be 155.55 mg GAE/kg, with a minimum of 35.36 mg GAE/kg, and a maximum of 365.94, while the mean total flavonoid content was found to be 22.80 mg

QE/kg, with a minimum of 4.80 mg QE/ kg, and a maximum of 54.78 mg QE/kg.

http://www.jaas.org.uk/articles/Total-polyphenol-content-of-... 10.10.2011

Keywords

Pine honey, Total flavonoid content, Total phenolic acid, Muğla.

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