doi:
e-ISSN: 2687-6698
Research Article
Turkish Journal of Analytical Chemistry
TurkJAC
www.turkjac.org
2019

HPLC analyses of polyphenolic compounds in oak (*Querces frainetto*) honey from Kırklareli region of Turkey

Yakup Kara[1], Zehra Can[2]*, Sevgi Kolaylı[1]

[1]Karadeniz Technical University, Faculty of Sciences, Department of Chemistry, 61080, Trabzon, Turkey

[2]Bayburt University, Faculty of Applied Sciences, Department of Emergency Aid and Disaster Management, 69000, Bayburt, Turkey

Abstract

In this study, phenolic profile and total phenolic contents of oak honey, a kind of dark honeydew honey, were determined. The methanolic honey extract was enriched by liquid-liquid extraction with diethyl ether and ethyl acetate, and then was analyzed by RP-HPLC-UV with acetonitrile: water mobile phase. Nineteen phenolic standards were used to prepare calibration graphics. Seven phenolic acid (gallic acid, protocatequic acid, *p*-OH benzoic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid) and twelve flavonoids (catechin, epicatechin, rutin, myricetin, resveratrol, daidzein, luteolin, *t*-cinnamic acid, hesperetin, chrysin, pinocembrin, caffeic acid phenlyester (CAPE) were used. Total phenolic contents of the honey were measured by Folin Ciocalteau's assay. All of the polyphenols except epicatechin, rutin, luteolin, and hesperetin were detected in varying amounts. Protocatequic acid, ferulic acid, myricetin, and chrysin were the most abundant phenolic compounds. Total phenolic contents of the honey were from 54 to 88 mg GAE/100g. In summary, oak honey has high apitherapeutic value with rich polyphenol diversity.

Keywords: Oak honey, antioxidant, phenolic, HPLC, Folin Ciocalteau's

1. Introduction

Honey is a natural product and is defined as a functional food. Its composition and biologically active properties depend on the flora and climate of the region where it is produced. According to the sources of production are collected in two classes as blossom and dew honey. Although blossom honey is collected from the flower nectars, dew or secretion honey are made up of different sugar extracts of sweating or insect secretions on trees and leaves [1]. There are two different honeydew honey; one of them is insect secretion honey such as pine honey, and other is a kind of infiltration or percolation some sweet mixtures from leaves, seeds, stems of threes like oak honey [2].

Turkey's seven geographical regions contain many different oak forests, but the Kırklareli region is the most oak honey production area of Turkey. This region has suitable climatic conditions for oak honey production. Oak honey is also known as manna honey and is produced second half of August. In the rainy season, this honey production is reduced. This honey has high viscosity is a dark color, caramelized taste, characteristic smells and non-crystallized. In many studies was determined oak honey has the high antioxidant capacity and that this attribute of polyphenols that contains [2,3]. For the determination of individual phenolic compounds, it is generally necessary their isolation from the sample matrix, then, the identification and the quantification steps. Thus, in this study, we aimed to determine the antioxidant and phenolic profile of oak honey which is rich in polyphenol in the Kırklareli region.

Citation: Y. Kara, Z. Can, S. Kolaylı, HPLC analyses of polyphenolic compounds in oak (*querces frainetto*) honey from Kırklareli region of Turkey, Turk J Anal Chem, 1, 2019, 17-20.

***Author of correspondence**: e-mail: zehracan61@gmail.com
Tel: +90 505 8860607
Received: November 12, 2019
Accepted: November 25, 2019

2. Experimental

The reagents used were of analytical grade. All phenolic standards were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemie GmbH (Germany). Folin–Ciocalteu's phenol reagent and TPTZ were obtained from Fluka Chemie GmbH (Switzerland). Trolox was purchased by AppliChem (Darmstadt, Germany).

Iron(III) chlorid hexahydrate ($FeCl_3.6H_2O$), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), iron(II) sulphate heptahydrate ($FeSO_4.7H_2O$), and DPPH (2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Glacial acetic acid, sodium acetate, and ferric chloride were supplied from Merck. Sartorius Minisart RC 15 LC syringe filters (RC-membrane, 0.45 μm), Sartorius (Darmstadt, Germany) were employed.

2.1. Samples

Six honey samples were obtained from the Kırklareli Beekeepers Union in 2019 and stored at dark room temperature until analysis was performed.

2.2. Honey extraction for antioxidant activity and phenolic analysis

Approximately 10 g of each honey was added to an equal volume (50 mL) of 100% methanol and the mixture was continuously stirred with a Heidolph Promax 2020 shaker (Schwabach, Germany) at room temperature for 24 h. Then, particles were removed by using filter paper. The final volume of the obtained solution was set with 100% methanol. The methanolic extract was divided into two parts to make antioxidant tests and phenolic compound analysis.

The methanolic extract was completely evaporated at 40°C using a rotary evaporator. The residue was dissolved in 10 mL of distilled water adjusted to pH 2. Liquid-liquid extraction was applied with 5×3 mL diethyl ether and 5×3 mL ethyl acetate, consecutively [4]. Both diethyl ether and ethyl acetate phases were collected and the solvents were completely removed by rotary evaporation (IKA-Werke, Staufen, Germany) at 40°C. To resuspend the pellet, 2 mL methanol was used.

For HPLC analysis, the suspension was filtered with syringe filters (RC-membrane, 0.45 μm) and injected to the HPLC system.

2.3. Total phenolic content (TPC)

The total phenolic content of samples was determined by Folin-Ciocalteu reagent and some modifications were made according to the Slinkard and Singleton methods [5]. Firstly, 400 μL of distilled water of both sample solution and standard solution after, 400 μL of 0.5 N Folin-Ciocalteu reagent was added. After 20 μL sample solution in all samples and 20 μL gallic acid in standard solution was added and then vortexed. After 400 μl of 7.5% of Na_2CO_3 was added and then vortexed then incubated for 2 hours at room temperature, absorbance measured at 760 nm using gallic acid as a standard. The results were given as mg gallic acid equivalents per 100 g sample (GAE mg/100g).

2.4. Total flavonoid content (TFC)

Total flavonoid content was determined by a colorimetric method as described previously Fukumoto and Mazza [6]. Firstly, 0.25 mL each sample solution, and 2.15 mL methanol 0.05 mL of 10% $Al(NO_3)_3$ and 0.05 mL of 1 M $NH_4CH_3CO_2$ was added to a test tube then was mixed well incubated at room temperature for 40 minutes. Then, the absorbance was measured against the blank 415 nm. The results were expressed as mg quercetin equivalents (QE) per 100 g sample (mg QE/ 100g).

2.5. Ferric reducing/antioxidant power (FRAP) assay

The total antioxidant potential of each sample solution was determined using the ferric reducing ability of FRAP assay by Benzie and Strain [7] as a measure of antioxidant power. Briefly, the FRAP reagent was prepared by mixing an acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl, and 20 μM $FeCl_3$. The sample of 50 μL and the FRAP reagent of 1.5 mL were well mixed. The absorbance was taken at 593 nm after 4 min. The standard curve was prepared using different concentrations of $FeSO_4.7H_2O$ and the results were expressed as μmol $FeSO_4.7H_2O$/g).

2.6. Analysis of phenolic compounds by HPLC-UV

The samples and standards qualitative analysis and quantitative determination of particular components of the fractions were analysed using high-performance liquid chromatography (HPLC) (Elite LaChrom Hitachi, Japan) with a UV detector. The separation was done on a column with a reversed-

phase C18 column (150 mm×4.6 mm, 5µm; Fortis), in gradient solvent systems A (2% AcOH in water) and solvent B (70:30, acetonitrile/water) which was sonicated before stirring and continuously degassed by the built-in HPLC system. The flow rate was kept constant at 1 mL min^{-1} using gradient programming; starting the flow of mobile phase as B (5%) to three minutes, gradually increasing (up-to 15, 20, 25, 40 and 80% at 8, 10, 18, 25 and 35 minutes respectively) and decreasing to 5 % at 40 minutes and left for 10 minutes to equilibrate in the column. The phenolic profile was determined according to Cakır [8].

3. Results and discussion

Flower honey is derived from honeybees are collecting nectar from plants, whereas honeydew is derived from honeybees collected sweet substances mainly from the excretions of plant-sucking insects (Hemiptera) on the living parts of plants or secretions of the living parts of plants.

In this study, was determined antioxidant and phenolic compounds of oak honey are honeydew, honey. Three different methods were utilized to evaluate the antioxidant capacity of the honey; TPC, TFC and the ferric reducing antioxidant assay (FRAP) reflecting total antioxidant capacity. TPC of the kinds of honey varied widely, from 44.75 to 75.58 mg GAE/100 g sample (Table 1).

Table 1. Total phenolic, total flavonoid and FRAP of oak honey.

Samples	TP mg GAE/100 g sample	TF mg QE/100 g sample	FRAP (µmol $FeSO_4.7H_2O$/g sample)
H1	44.75 ± 0.01	-	4.16 ± 0.08
H2	54.59 ± 0.02	0.06 ± 0.01	5.06 ± 0.08
H3	59.30 ± 0.01	1.16 ± 0.02	4.64 ± 0.06
H4	72.06 ± 0.01	1.94 ± 0.04	6.40 ± 0.01
H5	75.58 ± 0.01	1.99 ± 0.06	3.20 ± 0.03

TFC of the honey varied widely, from 0.06 to 1.99 mg QE/100 g sample. Frap results of the samples ranged from 3.20 to 6.40 µmol $FeSO_4.7H_2O$/g. Antioxidant compounds in honey samples provided from different climates reflect the floral origin and the biological quality [9]. It is reported that ark amber-colored honey such as oak honeydew honey and chestnut honey have a high antioxidant capacity, resulting from their phenolic compositions [10]. Other studies have also reported that oak honey total phenolic contents of 36.81-62.26 mg GAE/100g [11]. The results are similar to the results of our study. The total polyphenolic content of Anzer honey, light-colored honey, was found 19.50 to-38.30 mg GAE/ 100 g honey in our other studies [12]. According to these results, it was reported that dark honey contains higher phenolic substances and associated antioxidant activity than light-colored honey [11,13,14].

The true quality and, of course, the biological active value of honey is due to the various secondary metabolites present in the structure rather than the sugars it contains [15]. These secondary metabolites have not only anti-oxidant activities, but also anti-microbial, anti-tumoral, and anti-inflammatory functions [11-16].

The phenolic compounds present in the honey were determined by RP-UV-HPLC. In this study, nineteen phenolic standards were used (Figure 1).

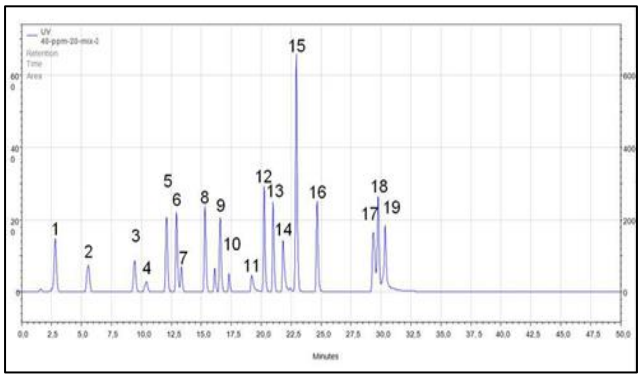

Figure 1. HPLC-UV chromatogram of phenolic standards 1. Gallic acid, 2. Protocatequic acid, 3. p-OH Benzoic acid, 4. Catechin, 5. Caffeic acid, 6. Syringic acid, 7. Epicatechin, 8. p-Coumaric acid, 9. Ferulic acid, 10. Rutin, 11. Myricetin, 12. Resveratrol,13. Daidzein, 14. Luteolin, 15. t-Cinnamic acid, 16. Hesperidin, 17. Chrysin, 18. Pinocembrin, 19. CAPE

The phenolic component results of honey are as in Table 2. All standards except for luteolin, epicatechin and hesperidin were detected at different concentrations. Our previous study determined that catechin, vanillic acid, syringic acid, daidzein, and luteolin were not detected in any specimens [3].

In this study was not detected luteolin in any honey. In our findings, protocatechuic acid, ferulic acid, myricetin, and chrysin have been reported as major phenolic compounds in oak honey. In our previous study was found protocatechuic and ferulic acid as major phenolic compounds in oak honey [3].

Table 2. Phenolic profiles of the oak honeys.

Standards	H1	H2	H3	H4	H5
	(μg extract/g sample)				
Gallic acid	1.04	1.85	1.55	3.33	0.34
Protocateuic acid	5.07	8.20	12.74	14.83	5.64
p-OH Benzoic acid	2.70	2.02	0.89	1.32	4.69
Catechin	0.89	n.d.	n.d.	n.d.	1.28
Caffeic acid	0.92	0.49	0.77	0.98	1.84
Syringic acid	0.59	0.26	0.53	0.67	1.36
Epicatechin	n.d.	n.d.	n.d.	n.d.	n.d.
p-Coumaric acid	0.63	0.86	0.48	0.62	1.50
Ferulic acid	1.75	4.89	2.96	5.03	0.39
Rutin	n.d.	n.d.	n.d.	n.d.	6.47
Myricetin	7.42	4.33	3.70	3.10	9.89
Resveratrol	1.25	0.56	0.61	0.76	0.79
Daidzein	2.04	0.87	0.75	0.98	2.16
Luteolin	n.d.	n.d.	n.d.	n.d.	n.d.
t-Cinnamic acid	0.02	0.09	0.21	0.42	0.11
Hesperidin	n.d.	n.d.	n.d.	n.d.	n.d.
Chrysin	2.68	1.70	2.28	2.81	3.32
Pinocembrin	2.00	0.28	1.21	1.81	2.94
CAPE	0.68	0.24	0.33	0.74	1.30

n.d.: not detected

Oak honey samples provided from the same botanical origins and biogeographical areas of production were analyzed. In this study, 5 oak honey samples collected from the Thrace region of Turkey exhibited honeydew honey characteristics, depending on their antioxidant activities and phenolic profiles. The oak honey had higher antioxidant activity and most phenolic compounds than light-colored honey. It is expected that the results will contribute to a more accurate evaluation of oak honey in the literature.

References

[1] S. Simova, A. Atanassov, M. Shishiniova, V. Bankova, A rapid differentiation between oak honeydew honey and nectar and other honeydew honeys by NMR spectroscopy, Food Chem, 134(3), 2012, 1706-1710.

[2] A. Ozkok, K. Sorkun, Some characteristic features of oak (quercus frainetto ten.) Fresenius Environ Bull, 27, 2018, 8359-8366.

[3] S. Kolayli, Z. Can, H. E. Çakir, O. T. Okan, O. Yildiz, An investigation on Trakya region oak (Quercus spp.) honeys of Turkey: Their physico-chemical, antioxidant and phenolic compounds properties, Turk J Biochem, 43(4), 2018, 362-374.

[4] K.H. Kim, R. Tsao, R. Yang, S.W. Cui, Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions, Food Chem, 95(3) 2006, 466-473.

[5] K. Slinkard, V.L. Singleton, Total phenol analyses: Automation and comparison with manual methods, Am J Enol Viticult, 28, 1977, 49-55.

[6] L.R. Fukumoto, G. Mazza, Assessing antioxidant and prooxidant activities of phenoliccompounds, J Agr Food Chem, 48, 2000, 3597-3604.

[7] I.F.F. Benzie, J.J. Strain, Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration, Method Enzymol, 299, 1999, 15-27.

[8] H.E. Çakır, Y. Şirin, S. Kolaylı, Z. Can, Validation methods for phenolic components with RP-HPLC-UV in various bee products, Apiterapi ve Doğa Dergisi, 1(1), 2018, 13-19.

[9] O. Muñoz, S. Copaja, H. Speisky, R.C. Peña, G. Montenegro, Contenido de flavonoides ycompuestos fenólicos de mieles chilenas e índice antioxidante, Quim. Nova, 30, 2007, 848-851.

[10] [9] M. C. Seijo, O. Escuredo, M. S. Rodríguez-Flores, Physicochemical properties and pollen profile of oak honeydew and evergreen oak honeydew honeys from Spain: A comparative study, Foods, 8(4), 2019, 126.

[11] H. Sahin, Honey as an apitherapic product: its inhibitory effect on urease and xanthine oxidase, J Enzyme Inhib Med Chem, 31(3), 2016, 490-494.

[12] M. Malkoç, H.E. Çakır, Y. Kara, Z. Can, S. Kolaylı, Phenolic composition and antioxidant properties of Anzer honey from black sea region of Turkey, Uludag Arıcılık Dergisi, doi.org/10.31467/uluarıcılık. 2019, 602906.

[13] [12] A.M. González-Paramás, R.J. García-Villanova, J.A.G. Bárez, J. S. Sánchez, R. A. Albajar, Botanical origin of monovarietal dark honeys (from heather, holm oak, pyrenean oak and sweet chestnut) based on their chromatic characters and amino acid profiles, Eur Food Res Technol, 226(1-2), 2007, 87-92.

[14] Z. Can, O. Yildiz, H. Sahin, E.A. Turumtay, S. Silici, S. Kolayli, An investigation of Turkish honeys: Their physico-chemical properties, antioxidant capacities and phenolic profiles, Food Chem, 180, 2015, 133-141.

[15] M. Malkoç, Y. Kara, A. Özkök, Ö. Ertürk, S. Kolaylı, Karaçalı (*Paliurus spina-christi* Mill.) balının karakteristik özellikleri, Uludag Arıcılık Dergisi. 19(1), 2019, 69-81.

[16] O. Yıldız, Z. Can, Ö. Saral, E. Yuluğ, F. Öztürk, R. Aliyazıcıoğlu, S. Kolaylı, Hepatoprotective potential of chestnut bee pollen on carbon tetrachloride-induced hepatic damages in rats, Evid-Based Complementary Altern Med, 2013, 461478.