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INVESTIGATION OF PHENOLIC COMPOSITION OF ORGANICALLY-GROWN STRAWBERRY AND BLUEBERRY

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ABSTRACT
The present study aimed to investigate phenolic compounds of strawberry which is organically-grown in Bursa region and blueberry in Trabzon region. The phenolic acids and flavonoids were detected by RP-HPLC according to their retention times with the help of diode array detector at 280 nm, 320 nm and 360 nm. Investigated compounds were gallic acid, epigallocatechin, catechin, epicatechin, epicatechin gallate, 2 coumaric acid, 3 coumaric acid, 4 coumaric acid, chlorogenic acid, cafeic acid, neochlorogenic acid, trans resveratrol, ferulic acid, quercetin, kaempferol and myricetin. According to results it was determined that bluberry fruit contains high amounts of chlorogenic acid (644.25 mg/kg) and among the phenolic acids in strawberries, 4 coumaric acid is regarded as major phenolic compound (3.76 mg/kg). The highest level of myricetin compounds was measured in blueberry (14.41 mg/kg), on the contrary, myricetin was not identified in strawberry fruits but this fruit is especially rich in catechins (58.34 mg/kg).
Keywords: Phenolics, bioactivity, antioxidant, strawberry, blueberry, organic

ORGANİK OLARAK YETİŞTİRİLEN ÇİLEK VE YABANMERSİNİ MEYVELERİNİN FENOLİK BİLEŞEMİNİN İNCELENMESİ

ÖZ
Bu çalışmada, Bursa yöresinde organik olarak yetişen çilek ve Trabzon yöresinde organik olarak yetiştişen yaban mersini meyvelerinin fenolik bileşenlerinin araştırılması amaçlanmıştır. Fenolik asitler ve flavonoidler, RP-HPLC tarafından tutma sürelerine göre 280 nm, 320 nm ve 360 nm'de DAD detektörü yardımıyla tespit edilmiştir. İncelenen bileşikler, gallik asit, epigallokateşin, kateşin, epikateşin, epikateşin gallat, 2 kumarik asit, 3 kumarik asit, 4 kumarik asit, klorogenik asit, kafeik asit, neoklorogenik asit, trans resveratrol, ferulik asit, kuarsetin, kamferol ve mirisetin'dir. Elde edilen sonuçlara göre, yaban mersini meyvesinin yüksek miktarda klorogenik asit (644.25 mg/kg) içerdiği ve çileklerde fenolik asitler arasında 4 kumarik asidin başlıca fenolik bileşik (3.76 mg/kg) olduğu belirlenmiştir. En yüksek mirisetin miktarı yaban mersini meyvesinde (14.41 mg/kg) olarak ölçülmüş, tam aksine çilek meyvelerinde mirisetin saptanmamıştır, ancak bu meyve özellikle kateşinler bakımından zengindir (58.34 mg / kg).
Anahtar kelimeler: Fenolikler, biyoaktivite, antioksidant, çilek, yaban mersini, organik

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INTRODUCTION

In recent years, the increasing interest for human health, nutrition and the prevention from illness has driven the demand of the consumer to foods and quality raw material with high nutraceutical value (Cecilia *et. al.*, 2013). As a part of a diet, consumption of certain fruits may have noticeable long-term physiological effects. Among different fruit species, berries have attracted a great attention for their bioactivity and they are part of human diet all over the world (Amakura, Umino, Tsuji, & Tonogai, 2000). Especially strawberry and blueberry, belong to the best dietary sources of bioactive compounds (BAC) such as anthocyanins, flavons and other phenolic compounds. They have received increasing attention because of their remarkable antioxidant capacity. BAC compounds are responsible for various health benefits of berries, such as prevention of inflammation disorders, cardiovascular diseases or protective effects to lower the risk of various cancers. (Bomser *et al.*, 1996; Heinonen *et al.*, 1998).

Organic cultivation methods are gaining popularity because of the concerns over environmental contamination and health benefits (Bourn & Prescott, 2002). Researches have reported that organic fruits and vegetables have higher levels of flavonoids. (Asami, Hong, Barrett & Mitchell, 2005; Mitchell *et al.,* 2007). In addition, higher levels of anthocyanins and phenolic compounds, and higher antioxidant capacities were found in organically cultivated blueberries (Wang, Chen, Sciarappa, Wang & Camp, 2008). Moreover, a higher antiproliferative activity towards cancer cells was found in extracts from organically grown strawberries than conventionally grown (Olsson, Andersson, Oredsson, Berglund & Gustavsson, 2006). Turkey ranks third in the world the production of strawberry (FAO, 2017). The strawberry is considered a functional food that offers multiple health benefits, including antioxidant, cardiovascular, antihypertensive, and antiproliferative effects (Basu, Nguyen, Betts & Lyons, 2014). The main polyphenol compounds described in strawberries are anthocyanins, flavan-3-ols, ellagitannins, glycosides of quercetin and kaempferol (Aaby, Mazur, Nes & Skrede, 2012; Määtta, Kamal- Eldin, Kaisu & Törronen, 2004).

It is well documented that blueberries are rich in phenolics, particularly hydroxycinnamic and hydroxybenzoic acids and their derivatives such as chlorogenic, caffeic, gallic, p-coumaric, ferulic, ellagic, syringic, vanillic acids (Hakkinen & Torronen, 2000; Mattila, Hellstrom & Torronen, 2006; Rodriguez-Mateos, Cifuentes-Gomez, Tabatabaee, Lecras & Spencer, 2012). On the other hand, recent studies indicated that the contents of anthocyanins, chlorogenic acid and quercetin were highly dependent upon blueberry cultivar, while seasonal variations had a comparatively minor effect (Brambilla, Lo Scalzo, Bertolo & Torreggiani, 2008; Scalzo *et al.,* 2013). Chlorogenic acid is a colorless phenolic of blueberries formed by esterification of quinic acid with caffeic acids (Clifford, 1999) and also is the major phenolic acid in blueberries previously reported by Correa-Betanzo *et al.*, 2014. Catechin is another major phytochemical belonging to flavonoids found in berries. The most common dietary catechins are catechin, gallocatechin, epicatechin, epigallocatechin, epicatechin 3-gallate, and epigallocatechin 3-gallate (Arts *et al.,* 2000; Cieslik *et al.,* 2006).

The objective of this research was to determine the phenolic composition of strawberry and blueberry fruit species organically grown in Turkey (Bursa and Trabzon provinces) to provide information useful for planning effective antioxidant dietary consumption using local products.

MATERIALS AND METHODS

Fruit samples

Strawberry (*Fragaria×ananassa Duch*.) were harvested from Bursa province and blueberry (*Vaccinium corymbosum L.*) was harvested from Trabzon province. Berries at the full maturity stage were harvested from the beginning of August to the middle of September 2015. Samples were maintained fresh in an icebox during transport to the laboratory and subsequently stored in a refrigerated room at 4 ˚C; only undamaged fruits were selected and they were extracted and analysed within 24 h. Fruit extracts were used for the HPLC analysis of the phenolic composition.

Standards and chemicals

The following standards were used to determine phenolic compounds in analyzed fruits: caffeic acid trans resveratrol, chlorogenic acid from Dr. Ehrenstorfer (Augsburg, Germany); epigallocatechin, catechin, epicatechin, epicatechin gallat, 2 coumaric acid, 3 coumaric acid, 4 coumaric acid, , ferulic acid, kaempferol, myricetin from Extrasynthese (Genay Cedex, France).

The chemicals for mobile phases and sample preparation were methanol and acetic acid from Sigma-Aldrich. The water used in sample, standards and mobile phase preparation was double distilled and purified with a Purelab Option-Q water purification system by Elga (UK).

Extraction of phenolic compounds

The extraction of fruit samples was performed as described (Mulero *et al.,* 2010) with some modifications. Fresh berry samples were mechanically homogenised. A weighed quantity of homogenised sample (approximately 5 g) was mixed with 50 ml extraction solution (50% methanol/water) (v/v) during 16 hours and cooled ultrasonic bath for 30 min. After extraction, the fruit extracts were centrifuged for 25 min at 5400 rpm for 2 times. The supernatant was filtered through a 0.45 μm PVDF filter and transferred to a amber coloured vial prior to injection into the HPLC system.

Determination of phenolic acids and flavonoids

The HPLC analysis was performed an HPLC system with a PDA detector (Shimadzu, Japan). The column was an Inertsil ODS-3 (250 x 4.6 mm, 5 μm; GL Sciences Inc., Japan) operated at 40 ^{0}C. The elution solvents were methanol (A) and bi-distilled water containing 2% acetic acid (B). Samples were eluted according to the gradient described by Velioğlu (2007) with some modifications, with an injection amount of 20 μL and a flow rate of 1 mL/ min. Phenolic compounds of samples were eluted according to a linear gradient from 100% to 95% B in the first 3 min, followed by a linear gradient from 95% to 80% B for 15 min, then holding at 80% B an isocratic flow for 7 min, followed by a linear gradient from 80% to 75% B for 5 min, followed by a linear gradient from 75% to 60% B for 5 min, followed by a linear gradient from 60% to 50% B for 15 min, followed by a linear gradient from 50% to 40% B for 10 min, followed by a linear gradient from 40% to 100% B for 2 min, then an isocratic flow for 5 min before returning to the initial conditions. The gallic acid, epigallocatechin, catechin, epicatechin, epicatechin gallat, 2 coumaric acid, 3 coumaric acid, 4 coumaric acid were detected at 280 nm, chlorogenic acid, cafeic acid, neochlorogenic acid, trans resveratrol, ferulic acid were detected at 320 nm and quercetin, kaempferol, myricetin were detected at 360 nm according to their retention times. The retention times and wavelengths of phenolic compounds are presented in Table 1.

Statistical evaluation

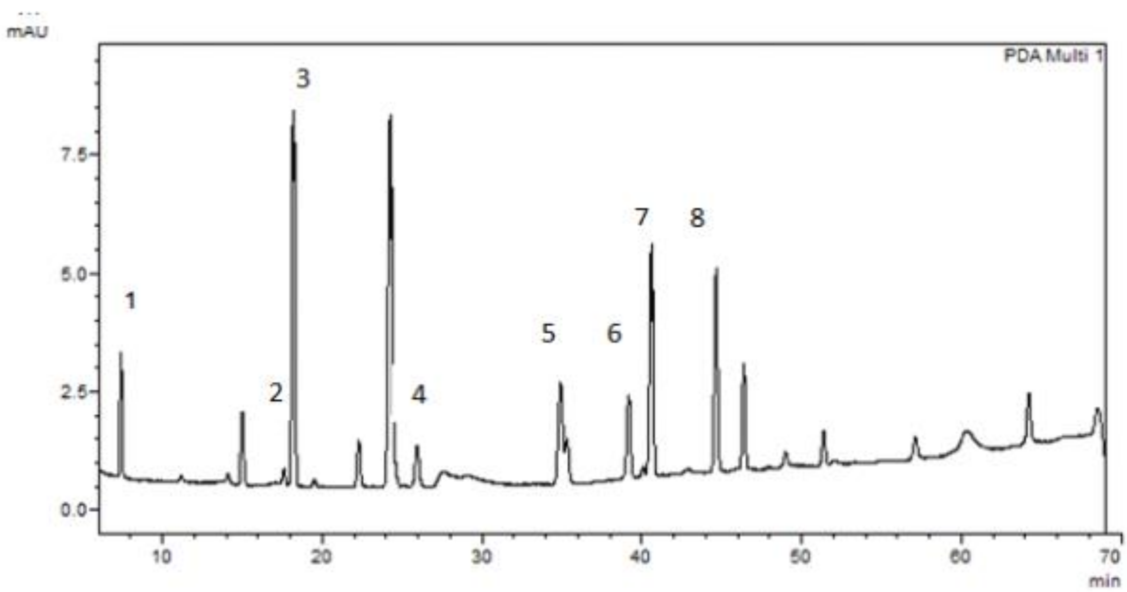
The results were analyzed using Microsoft Excel program. Means and standard errors of the phenolic content were calculated from three replicates (n=3) for each individual berry species.

RESULTS AND DISCUSSION

The phenolic compounds detected in strawberry and blueberry samples studied were classified into flavonoids and phenolic acids. Typical chromatograms of phenolic acid and flavonoid standards are given Fig. 1. Table 2. shows the phenolic acid content of strawberries and blueberries. According to results it was determined that blueberry fruit contains high amounts of chlorogenic acid (644.25 mg/kg fw) and among the phenolic acids in strawberries, 4 coumaric acid is regarded as major phenolic compound (3.76 mg/kg). Table 3. shows quantities of flavonoids determined in strawberry and blueberry. The highest level of myricetin compounds was measured in blueberry (14.41 mg/kg), on the contrary myricetin was not identified strawberry fruits but this fruit is especially rich in catechins (58.34 mg/kg).

Table 1. Retention time and wavelength of phenolic acids and flavonoids.

	Retention time (min.)	Wavelength (nm)	Abbreviation
Flavonoids			
Epigallocatechin	17.62	280	Epig
Catechin	18.20	280	Cat
Epicatechin	25.95	280	Epicat
Epicatechin gallat	35.33	280	Epic gal
Trans resveratrol	46.39	320	Trans res.
Myricetin	49.02	360	Myr
Quercetin	57.12	360	Quer
Kaempferol	64.22	360	Kaem
Phenolic acids			
Gallic acid	7.41	280	Gallic a
4 coumaric acid	34.92	280	4 coum. a
3 coumaric acid	40.61	280	3 coum. a
2 coumaric acid	44.65	280	2 coum. a
Neochlorogenic acid	15.00	320	Neochl. a
Chlorogenic acid	22.29	320	Chl. a
Cafeic acid	24.25	320	Caf. a
Ferulic acid	39.18	320	Fer. a



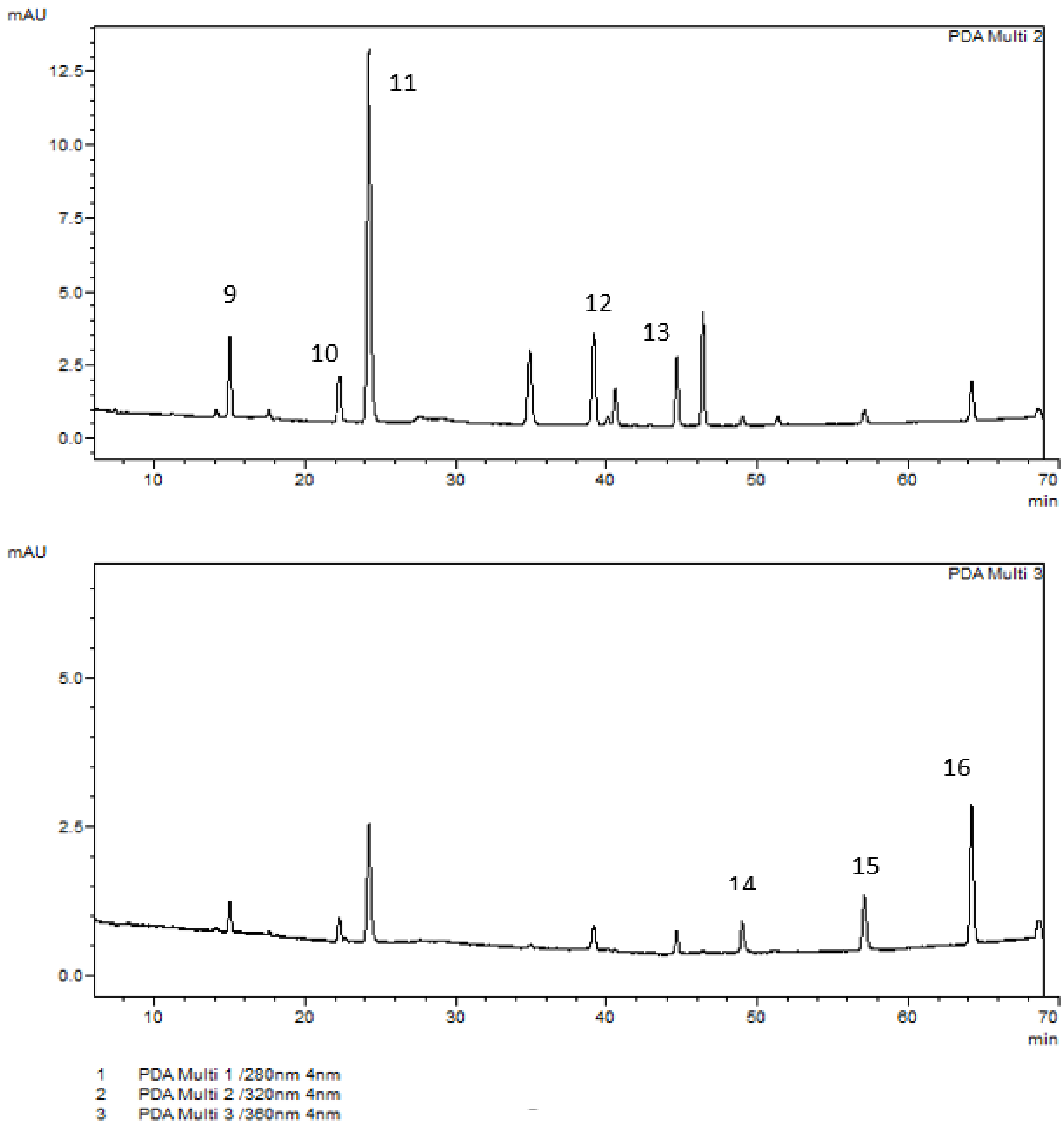


Fig. 1. Chromatograms of phenolic acid and flavonoid standards. Peak identification, 1:gallic a.; 2:epig.; 3:cat.; 4:epicat.; 5:4 coum. a.; 6:epic. gal.; 7:3 coum. a.; 8:2 coum. a.; 9:neochl. a.; 10:chl. a.; 11:cafeic a.; 12: ferulic a.; 13:trans res.; 14:myr.; 15: quer.; 16: kaem.

According to research of Grace *et al.* (2019), sixteen phenolic compounds in organically grown blueberry samples were successfully separated, identified and quantified, including flavonols, phenolic acids and trans resveratrol. Chlorogenic acid represented the primary phenolic acid in all blueberry varieties and this compound showed the highest level (1492-4150 mg/kg dw) and gallic acid amount was dedected 0.05-5.4 mg/kg dw, these results were in line with our results. In our study, clorogenic acid was detected as the major phenolic compound (3796.41 mg/kg dw) and gallic acid was determined 3.83 mg/kg dw (Table 2). For the other investigated phenolic components catechin was detected 117.5-259.9 mg/kg dw, epicatechin 26.0-109.9 mg/kg dw, quercetin 5.6-15.4 mg/kg dw and caffeic acid 23.7-35.8 mg/kg dw (Grace *et. al,* 2019). Their results are not accordance with our study. Cardeñosa *et al.* (2016) one of the researchers who studied on this subject found the chlorogenic acid values between 4890 and 7730 mg/kg dw. However, Kraujalyte˙ *et al.* (2015) determined this value as 20-346.8 mg/kg fw in fresh berries. The values obtained in our study were 644.25 mg/kg fw (Table 2). Yıldız *et al.* (2015) found that gallic acid content between nd-8.6 mg/kg fw, caffeic acid 2.0-121.6 mg/kg fw, coumaric acid 0.9-2.0 mg/kg fw, catechin 11-29.9 mg/kg fw, epicatechin 11.5-161. 4 mg/kg fw, resveratrol 2.9-164.0 mg/kg fw, myricetin 7.6-212.3 mg/kg fw, quercetin 0.6-28.7 mg/kg fw and kaempferol 0.6-11.4 mg/kg fw. The results of gallic acid, caffeic acid, myricetin, and quercetin were parallel with our findings.

These differences may be originate from cultivar, growing techniques, time of harvest, composition of the fertilizers.

Table 2. Quantities (mg/kg fw) of phenolic acids determined in strawberry and blueberry

	Phenolic acids							
	Gallic a.	2 coum. a.	3 coum. a.	4 coum. a.	Chl. a.	Caff.a.	Neochl. a.	Fer. a.
Strawberry *	1.85±0.29	0.35±0.06	ND	3.76 ±0.33	0.22±0	ND	0.09±0.05	0.12±0.06
Blueberry**	0.65±0.09	ND	ND	ND	644.25±3.96	4.16±0.16	4.66±0.15	0.63±0.08

Abbreviations and the full names for phenolic compounds are presented in Table 1. Means and standard errors of the phenolic content were calculated from tree replicates (n=3) for each individual berry species.
*****Dry matter : 11.05 (%)
**Dry matter:16.97 (%)

Table 3. Quantities (mg/kg fw) of flavonoids determined in strawberry and blueberry

	Flavonoids							
	Trans res.	Quer.	Myr.	Kaem.	Cat.	Epicat.	Epic. gal.	Epig.
Strawberry *	1.95±0.19	0.65±0.27	ND	0.27±0.12	58.34±0.07	6.99±1.27	ND	5.92±0.74
Blueberry**	ND	0.58±0.07	14.41±0.34	0.33±0.04	ND	0.66±0.07	ND	ND

Abbreviations and the full names for phenolic compounds are presented in Table 1. Means and standard errors of the phenolic content were calculated from tree replicates (n=3) for each individual berry species.
*****Dry matter : 11.05 (%)
**Dry matter:16.97 (%)

Fernández *et al.,* (2014) found remarkably high flavanol content in strawberries, especially for (+) catechin (123.7–211.8 mg/ kg fw and 40.1–227.4 mg/ kg fw), in 2011 and 2012 seasons, respectively. And also they detected gallic acid amount as 15.6-24.2 mg/kg fw, ferulic acid 1.5-5.1 mg/kg fw, caffeic acid nd-48.2 mg/kg fw, coumaric acid 0.9-2.7 mg/kg fw, kaempferol 0.53-1.04 mg/kg fw and trans resveratrol nd-1.23 mg/kg fw. In our study Caffeic asid (nd), ferulic acid 0.12 mg/kg fw , gallic asid 1.85 mg/kg fw and coumaric acid (0.35-3.76 mg/kg fw) was dedected in strawberry fruits (Table 2). A just compound coumaric acid were higher than from the researcher results but other copenents lower than in our study. The predominant flavonols in our samples was catechin (58.34 mg/kg fw) in contrast to data reported by other authors (Aaby, Ekeberg *et al.,* 2007; Buendia *et al.,* 2010; Määtta *et al.,* 2004; Da Silva Pinto, Lajolo, Genovese, 2008;

Del Bubba *et al.,* 2012), who found quercetin derivatives to be the main flavonol in strawberries. Our results were similar to those obtained by this research compared with reported values: 25–81 mg/kg fw (Aaby *et al.,* 2007, Arts *et al.,* 2000).

As a result, these differences of composition and amount of phenolic compounds might be explained by cultivation techniques, variety, harvest season, agroclimatic conditions and fertilization programme and organically grown fresh blueberry fruits phenolic compounds content were higher than organically grown fresh strawberry fruits in terms of the inspected sixteen components.

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