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Phenolic compounds and phytochemicals in fruits of black mulberry (*Morus nigra* L.) genotypes from the Aegean region in Turkey

Volkan Okatan

Sivaslı Vocational High School
Uşak University
64800 Sivaslı, Uşak/Turkey

ABSTRACT

Turkey is one of the most important centres of mulberry genetic resources and mulberries grow naturally almost everywhere in Turkey. This study was carried out to determine the most important phenolic compounds and phytochemical properties of mulberry (*Morus nigra* L.) genotypes collected from natural resources in 2016-2017 in the province of Uşak (Turkey). The investigated biochemical characteristics included: total soluble solids content (TSS), pH, titratable acidity, total phenolic content, vitamin C and antioxidant capacity (DPPH). The highest values of phenolic compounds, i.e. ellagic acid, rutin, quercetin, gallic acid, catechin, chlorogenic acid and caffeic acid, were as follows: 5.89, 133.60, 11.25, 40.90, 10.54, 97.59 and 21.93 g 100 g^{-1}, respectively. The highest values of total phenolics, vitamin C content and antioxidant capacity determined in the investigated mulberry genotypes were 2977.30 mg GAE g^{-1}, 31.34 mg 100 g^{-1} and 26.80%, respectively. The genotypes 64USA08, 64USA06 and 64USA10 can be recommended in terms of the most valuable chemical composition and used for future breeding purposes. It is desirable to take steps to implement an intensive programme for the preservation of *Morus nigra* L. biodiversity in Turkey.

Keywords: antioxidant activity, chemical composition, organic acids

INTRODUCTION

Fruits are a large horticultural group and include a lot of species and numerous cultivars, genotypes, accessions, etc., occurring in most parts of the world as cultivated, semi-wild and wild. All these three groups are important genetic resources of biodiversity, which support the life system on earth (Kamiloglu et al., 2009; Tosun et al., 2009; Ercisli et al., 2012; Canan et al., 2016; Zorenc et al., 2016; Caliskan et al., 2017).

The black mulberry (*Morus nigra* L.) is a member of the Moraceae family and is found in nature in many parts of the world. The tree can reach 10-13 metres in height (Rahman and Khanom, 2013). Dark and red fruits contain very important chemical substances in terms of human health, as well as disease prevention and treatment.

The *Morus nigra* L. species exists almost everywhere in Anatolia and is represented by wild-elderly trees (Gundogdu et al., 2017). The height of mulberry trees is 3-15 m, and they have a splayed, rounded, clustered crown structure. Branches are more frequent and shorter than in other mulberry species. The branch system is of medium strength. The main branches, one-year-old and two-year-old branches show narrow, moderate and strong outlets.

Corresponding author.
e-mail: okatan.volkan@gmail.com (V. Okatan).

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It is seen to be grey-brown colour in one year two years and one year old (Koyuncu and Vural, 2003).

The dark fruits are a unique source of phytochemicals and phenolic compounds. Fruits of *Morus nigra* L. are slightly acidic and quite dark in colour, correlated with a high anthocyanin content, which plays a significant role in pharmacological and food industries (Özgen et al., 2009). These anthocyanins were found to be inhibitory to the spreading of liver cancer cells in the human body (Karadeniz and Şişman, 2003). It is reported that mulberry fruit, molasses, compote and jam could be effective in the treatment of coronary failure and gastric and intestinal diseases (Rodriguez-Mateos et al., 2014).

Fruits of white and black mulberry contain sugars, organic acids (citric, malic, etc.), mucilage, tannin, dye (cyanine), pectin and vitamin C (Karadeniz and Şişman, 2003). The antioxidant and chemical contents of fruits are affected by many agents; in particular, ecological conditions and genotype characteristics have a significant impact on the diversification of these chemicals contents in black mulberry (Mikulic-Petkovsek et al., 2012; Sanchez et al., 2014).

The concentration of organic acids in mulberry fruits is highly variety-depended. The quantity of organic acids directly affects the taste due to the acid-sugar balance. The organic acids have been revealed to be in the form of free compounds, salts, esters, and glycosides. The organic acids are quickly oxidized in the human body, so they do not impact it unfavourably. Salts have a very important effect on the nutrition status of the human body since they increase the alkalinity level (Soyer et al., 2003). Malic, citric, and tartaric acids are the most common organic acids in fruits. Citric acid or malic acid are concentrated as the main acids in many fruits (Cemeroğlu et al., 2004).

Black mulberry in nature is very valuable for medicine and chemistry, gaining importance in the prevention and treatment of diseases. The aim of this study was to determine the most important physicochemical characteristics of thirteen mulberry (*Morus nigra* L.) genotypes from Turkey and to nominate those that were the most valuable for preservation, future investigations and breeding purposes.

MATERIAL AND METHODS

Study area

The province of Uşak is located in the Inner Aegean Region and has a climate structure in which various fruits may be grown successfully. In this regard, this region is a significant fruit growing area. The climate of the Uşak province shows a transition characteristic between the Aegean and Central Anatolian regions, but the continental climate prevails. The summers are hot, the winters are long and hard. The annual rainfall is between 430 mm and 700 mm. The temperature is between -24°C (winter) and +39.8°C (summer) (Polat et al., 2013).

Fruit material

Fruits of thirteen native black mulberry genotypes were collected in a distinct area of Uşak, Aegean Region, Turkey. The trees were close to being a hundred years old and naturally grown. Each identified genotype was named from 64USA01 to 64USA13. The gathering was performed in 2016 and 2017 when the fruits of the analyzed genotypes had reached the commercially mature stage. Three replicates including 50 uniform fruits of mulberry genotypes per repetition were used. The harvested fruits of the genotypes were then taken to the laboratory for analytical assays.

Total soluble solids content, pH and titratable acidity

Total soluble solids content (TSS) was determined with a digital refractometer (Model HI-96801 Hanna, German) at room temperature. The pH value was determined with a Hanna-HI 98103 pH meter; calibration was done using pH 4.0 and 7.0 buffer solutions. Titratable acidity was measured potentiometrically by titrating the sample with 0.1 NaOH until the pH reached 8.01, and was expressed as % citric acid equivalent.

Analysis of phenolic compounds

Phenolic compounds were detected in mulberry genotypes via the method of Rodriguez-Delgado et al. (2001). Extracts of genotypes were diluted with pure water in equal proportions. The prepared mixtures were centrifuged for 15 minutes at 15,000 rpm. The isolation of phenolic acids was carried out with an Agilent 1260 series HPLC system equipped with an on-line degasser (G 1322A), quat pump (G 1311A), autosampler (G 1313A), column heater (G 1316A), and UV detector (G 1315A). Instrument control and data analysis were carried out using Agilent HPLC Chemstation 10.1 edition with Microsoft Windows 2000.

Analysis of organic acids

Organic acids of mulberry genotypes were analyzed via the process of Bevilacqua and Califano (1989).

Juice extracts of mulberry fruit were obtained by crushing the fruits in tulle cloth; afterwards, the samples were kept at -20°C until analyzed. 5 mL of each sample was diluted with 20 mL of 0.009 N H_2SO_4 (Heidolph Silent Crusher M, Germany), then homogenized for one hour with a shaker (Heidolph Unimax 1010, Germany). The prepared mixtures were centrifuged for 15 minutes at 15,000 rpm, and the supernatants were strained with a 0.45 µm skin strainer followed by filtration through a thick filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), and run through a SEP-PAK C18 cartridge. Organic acid measurements were performed with Agilent 1260 series HPLC using an on-line degasser (G 1322A), quat pump (G 1311A), autosampler (G 1313A), column heater (G 1316A), and UV detector (G 1315A) at 214 and 280 nm wavelengths, controlled with the Agilent package program.

Analysis of DPPH radical scavenging activity

Determination of DPPH radical scavenging activity was performed by the method of Brand-Williams et al. (1995). The DPPH solution was freshly prepared before analysis. Then, 1 ml of 10^{-4} M DPPH in a methanol solution was taken and transferred to a glass tube coated with aluminium foil. 3 ml samples of the prepared 0, 3, 1.25, 6.25, 12.5, 25, 50, 100, 200, 400 µg ml^{-1} antioxidant solutions in methanol were added to the DPPH solution. Instead of the antioxidant solution, 3 ml of pure methanol was added to the control tubes. The samples were kept in the dark and room temperature for 30 minutes and then their absorbance was measured at 517 nm against methanol. Ascorbic acid and Trolox were used as standards (Somparn et al., 2007; Mishra et al., 2012). The percentage of DPPH scavenging activity was calculated using the following equation:

$$\% \text{ DPPH} = [(Ac - As)/Ac] \times 100$$

where Ac was the absorbance of the negative control (containing the extraction solvent instead of the sample) and As was the absorbance of the samples. The results were expressed as EC50 (µg ml^{-1}).

Determination of total phenolics

Total phenolics of mulberry genotypes were estimated by means of the Folin-Ciocalteu phenol reagent procedure (Singleton and Rossi, 1965). Absorbance was detected with a spectrophotometer (Jasco V-530) at 765 nm. The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per litre of extract.

Determination of vitamin C

After mashing and filtering the fruit of mulberry genotypes, juice samples were obtained. The juice samples were used for vitamin C analysis. The samples were homogenized by centrifuging, and 400 µL oxalic acid (0.4%) and 4.5 ml 2,6-diclorofenolindofenol solution were added to the supernatant. The data were read spectrophotometrically at a wavelength of 520 nm against a blank.

Determination of soluble sugars

A modified method of Melgarejo et al. (2000) was used for sugar (fructose, glucose and sucrose) analyses. 5 mL of fruit juice was centrifuged at 15,000 rpm for 2 minutes at a temperature of 4°C. The supernatants were passed through a SEP-PAK C18 cartridge. HPLC readings were obtained with µBondapak-NH2 column using 85% acetonitrile, as the liquid phase, with a refractive index detector (IR). Fructose and glucose standards were used for sugar estimations.

Statistical analysis

Descriptive statistics of the investigated parameters were represented as the mean ±SE. Experimental data were evaluated using analysis of variance (ANOVA), and significant differences among the means of three replicates ($p < 0.05$) were determined by Duncan's multiple range test using the SPSS 20 for Windows.

RESULTS AND DISCUSSION

Total soluble solids content, pH and titratable acidity

Total soluble solids content (TSS), pH and titratable acidity varied in all the genotypes at a statistically significant level, $p < 0.05$ (Tab. 1). In the studied genotypes, the highest TSS was measured in the fruits of 64USA12 genotype (19.43°Brix). The highest pH value was measured as 4.42 in 64USA07; the lowest value was in the 64USA04 genotype as 3.66. The titratable acidity content was determined to be between 1.47% (64USA11) and 1.97% (64USA10).

Erdoğan (2003) reported that TSS was 14.0-25.0% in the genotypes of *Morus nigra* L. in the Erzurum province, Turkey. Çam (2000) selected 25 mulberry species in a survey carried out in Edremit and Gevaş districts of the Van province. The mean pH values of the genotypes were 5.6-7.4, TSS values were found between 15.79-19.71%, and titratable acidity values between 0.17-0.30%.

Table 1. Total soluble solids content (TSS, °Brix), pH and titratable acidity values (%) of mulberry genotypes (mean for 2016 and 2017 ±SE)

Genotypes	TSS	pH	Titratable acidity
64USA01	14.23 ±0.07 m*	4.11 ±0.03 g	1.86 ±0.02 d
64USA02	15.94 ±0.08 j	4.16 ±0.02 f	1.89 ±0.08c
64USA03	18.84 ±0.09 c	4.23 ±0.03 e	1.93 ±0.06 b
64USA04	19.02 ±0.10 b	3.66 ±0.03 m	1.76 ±0.04 g
64USA05	16.34 ±0.08 i	3.73 ±0.03 l	1.80 ±0.05 f
64USA06	14.68 ±0.07 l	4.40 ±0.05 b	1.82 ±0.01 e
64USA07	16.97 ±0.08 g	4.42 ±0.01 a	1.64 ±0.02 j
64USA08	16.66 ±0.08 h	3.81 ±0.02 k	1.68 ±0.06 i
64USA09	17.86 ±0.09 e	3.86 ±0.04 j	1.72 ±0.03 h
64USA10	18.21 ±0.09 d	3.94 ±0.01 i	1.97 ±0.02 a
64USA11	17.38 ±0.09 f	3.99 ±0.05 h	1.47 ±0.02 m
64USA12	19.43 ±0.10 a	4.31 ±0.03 d	1.53 ±0.01 l
64USA13	15.76 ±0.08 k	4.38 ±0.01 c	1.52 ±0.01 k

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

Elmacı and Altuğ (2002), in their study of the taste characteristics of three mulberry varieties grown in the Aegean Region, determined the pH values of the varieties to be in the range 3.60-3.80 and the titratable acidity in the range of 1.51-1.79%. Çam and Türkoğlu (2004) conducted a study on the phenological and pomological characteristics and selection of Edremit and Gevaş mulberry genotypes. The researchers found that the mean pH ranged from 5.6 to 7.4, the TSS was from 15.79 to 19.71%, and the titration acidity varied from 0.163 to 0.264%. The average amounts of TSS in selected mulberry types ranged from 15.65 to 22.1%, pH 3.65-4.12, and titratable acidity ranged from 1.45 to 1.85 in a study carried out on *Morus nigra* L. grown in Bitlis (Okatan et al., 2016). In a study conducted on selected mulberry genotypes in the Antalya region, the amount of TSS in fruits was found to be 15-27%, pH value 3.74-5.65, titratable acidity 0.20-2.40% (Özdemir and Topuz, 1998). It is thought that these differences result from the differences in the genetic structures of mulberry genotypes, ecological factors, and differences in analysis techniques.

Total phenolics, vitamin C content and radical scavenging activity (DPPH)

Total phenolics, vitamin C content and radical scavenging activity (DPPH) were significantly differentiated in all the genotypes at a statistically significant level, $p < 0.05$ (Tab. 2). Differences were observed between the genotypes regarding the total phenolic content. The highest value of total phenolics was determined as 2977.30 mg GAE g^{-1} in the 64USA03 genotype, and the lowest value was determined as 1874.35 mg GAEg^{-1} in the 64USA10 genotype. Vitamin C content was the highest as 31.34 mg 100 g^{-1} in 64USA06 genotype, and the lowest value of 19.73 mg 100 g^{-1} was measured in the 64USA10 genotype. In the study, radical scavenging activity (%DPPH) was found to be in the range from 16.87 (64USA03) to 26.80 (64USA10).

Ten species of mulberry (*M. atropurpurea* Roxb) selected from China's Guandong region were investigated in a study by Huo (2004), who determined the mean vitamin C content as 10.02 mg 100 g^{-1}. Akbulut et al. (2006) determined the chemical, physicochemical properties and mineral salts distributions of 4 different mulberry species collected from the Gaziantep, Konya and Malatya regions. As a result of the study, the highest ascorbic acid content was found in red mulberry fruit (12.45 mg 100 g^{-1}). Total phenolic content was found between 114.3 and 354.5 mg 100 g^{-1}. Karacali (2012) stated that fruit species can be classified into three groups: poor, medium and rich in vitamin C, and in this respect the mulberry fruit is included in the group which is generally referred to as the middle group in terms of vitamin C content. Ercişli and Orhan (2008) reported that the vitamin C content in mulberry fruit of the genotypes grown in northeastern Turkey was in the range 14.9 to 18.8 mg 100 mL^{-1}. Ercisli and Orhan (2007) measured vitamin C content as 22.4, 19.4 and 21.8 mg 100 mL^{-1}, in white, red and black mulberries, respectively. Earlier reports had shown that antioxidant activities in *Morus nigra* L. were 15.037

Table 2. Total phenolics, vitamin C content and radical scavenging activity (DPPH) of mulberry genotypes (mean for 2016 and 2017 ±SE)

Genotypes	Total phenolics (mg GAE g^{-1})	Vitamin C (mg 100 g^{-1})	DPPH (%)
64USA01	2102.35 ±6.73 f*	21.46 ±0.11 g	18.92 ±0.11 f
64USA02	2784.45 ±8.91 b	20.46 ±0.10 j	18.35 ±0.11 g
64USA03	2977.30 ±9.53 a	21.03 ±0.11 i	16.87 ±0.10 k
64USA04	2028.25 ±6.49 g	21.35 ±0.11 g	18.25 ± 0.11 g
64USA05	2038.70 ±6.52g	29.31 ±0.15 b	25.06 ±0.15 b
64USA06	1943.70 ±6.22 j	31.34 ±0.16 a	17.98 ±0.11 i
64USA07	2106.15 ±6.74 f	22.17 ±0.11 f	18.96 ±0.11 f
64USA08	2008.30 ±6.43 h	21.14 ±0.11 h	18.07 ±0.11 h
64USA09	2388.30 ±7.64 d	25.14 ±0.13 d	21.49 ±0.13 d
64USA10	1874.35 ±6.00 k	19.73 ±0.10 k	26.80 ±0.16 a
64USA11	2494.70 ±7.98 c	26.26 ±0.13 c	22.45 ±0.13 c
64USA12	1997.85 ±6.39 i	22.13 ±0.11 f	19.29 ±0.12 e
64USA13	2143.20 ±6.86 e	22.56 ±0.11 e	17.49 ±0.10 j

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

-24.443 μM TE g^{-1} (Ozkaya, 2015). Total phenolic content in *Morus nigra* L. fruits was found between 1515-2570 GAE mg g^{-1} (Bae and Suh, 2007; Lin and Tang, 2007). The parallelism between present studies and cited references can conclude, thay chemical composition of mulberry species and genotypes is highly differentiated.

Phenolic compounds

Phenolic compounds in mulberry genotypes varied at a statistically significant level, $p < 0.05$ (Tab. 3). The highest concentrations of ellagic acid, rutin, quercetin, gallic acid, catechin, chlorogenic acid and caffeic acid were as follows: 5.89, 133.60, 11.25, 40.90, 10.54, 97.59 and 21.93 g 100 g^{-1}, and the lowest: 1.36, 32.06, 2.33, 21.83, 2.49,43.20 and 6.14 g 100 g^{-1}, respectively.

It has been shown that dark fruits such as mulberry are preferred by consumers due to their protective properties against some diseases (Ercisli and Orhan, 2008; Kostic et al., 2013). Gundogdu et al. (2011) and Natic et al. (2015) found chlorogenic acid and rutin as the main phenolic compounds. Gundogdu et al. (2011) determined the levels of gallic, catechin, chlorogenic, caffeic, syringic, p-coumaric, ferulic, coumaric, vanillic, and quercetin values in *Morus nigra* L. as 0.15, 0.08, 3.11, 0.13, 0.10, 0.13, 0.04, 1.42, and 0.11 mg g^{-1}, respectively. Memon et al. (2010), using three dissimilar extraction methods including

Table 3. Concentrations of phenolic compounds (mg 100 g^{-1}) of mulberry genotypes (mean for 2016 and 2017 ±SE)

Genotypes	Ellagic acid	Rutin	Quercetin	Gallic acid	Catechin	Chlorogenic acid	Caffeic acid
64USA01	5.40 ±0.10 c*	36.98 ±1.24 i	8.35 ±0.12 c	33.94 ±0.08 c	2.69 ±0.01 j	45.30 ±0.64 g	8.89 ±0.07 h
64USA02	2.77 ±0.03 h	133.60 ±1.06 a	6.97 ±0.03 f	24.42 ±0.76 i	10.54 ±0.06 a	43.76 ±0.16 h	17.97 ±0.03 b
64USA03	5.89 ±0.13 a	124.23 ±3.16 b	6.98 ±0.04 f	29.16 ±0.07 f	7.54 ±0.06 d	64.68 ±0.95 e	10.13 ±0.02 g
64USA04	5.64 ±0.07 b	85.63 ±1.43 d	7.35 ±1.03 d	26.75 ±0.82 g	4.04 ±0.01 h	97.59 ±0.41 a	10.06 ±0.03 g
64USA05	1.83 ±0.04 j	50.74 ±1.47 g	5.53 ±0.47 h	40.90 ±1.14 a	2.49 ±0.08 k	43.20 ±0.12 h	6.14 ±0.06 k
64USA06	1.38 ±0.06 k	32.06 ±0.23 k	2.98 ±0.12 j	32.63 ±0.03 d	9.92 ±0.03 b	78.27 ±0.42 d	11.72 ±0.01 e
64USA07	2.26 ±0.07 i	33.61 ±0.76 j	11.25 ±1.43 a	31.19 ±0.42 e	5.17 ±0.02 f	83.63 ±0.34 c	16.45 ±0.08 d
64USA08	3.27 ±0.03 f	42.69 ±0.89 h	7.17 ±0.23 e	23.79 ±0.04 j	5.57 ±0.03 e	92.81 ±0.16 b	7.25 ±0.05 j
64USA09	4.88 ±0.01 d	89.99 ±1.12 c	2.33 ±0.07 k	40.29 ±0.33 b	8.58 ±0.02 c	76.07 ±0.21 d	16.75 ±0.05 c
64USA10	4.46 ±0.08 e	67.51 ±0.72 f	8.56 ±0.04 b	25.86 ±0.06 h	4.10 ±0.01 h	90.52 ±0.06 b	21.93 ±0.03 a
64USA11	3.12 ±0.06 g	67.80 ±1.24 f	7.35 ±0.04 d	21.83 ±0.09 k	3.71 ±0.02 i	54.79 ±0.23 f	10.38 ±0.06 f
64USA12	1.36 ±0.01 k	37.12 ±0.32 i	6.89 ±0.35 g	34.52 ±0.06 c	4.61 ±0.01 g	48.72 ±0.24 g	16.49 ±0.02 d
64USA13	1.38 ±0.06 k	77.72 ±0.77 e	4.53 ±0.16 i	26.94 ±0.06 g	2.28 ±0.05 l	60.77 ±0.41 e	7.98 ±0.02 i

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

Table 4. Concentration of organic acids (g 100 g^{-1}) of mulberry genotypes (mean for 2016 and 2017 ±SE)

Genotypes	Oxalic acid	Citric acid	Tartaric acid	Malic acid
64USA01	0.84 ±0.06 c*	4.54 ±0.09 b	0.86 ±0.04 a	11.58 ±0.29 b
64USA02	0.58 ±0.04 g	2.33 ±0.05 g	0.38 ±0.02 f	9.44 ±0.24 c
64USA03	1.06 ±0.07 b	2.33 ±0.05 g	0.40 ±0.02 f	8.07 ±0.20 f
64USA04	1.04 ±0.07 b	2.00 ±0.04 i	0.51 ±0.03 d	8.35 ±0.21 e
64USA05	1.25 ±0.09 a	2.29 ±0.05 g	0.68 ±0.03 b	6.65 ±0.17 h
64USA06	0.61 ±0.04 f	3.49 ±0.07 d	0.54 ±0.03 c	7.43 ±0.19 g
64USA07	0.45 ±0.03 h	2.12 ±0.04 h	0.56 ±0.03 c	8.36 ±0.21 e
64USA08	0.77 ±0.05 d	2.14 ±0.04 h	0.22 ±0.01 h	13.65 ±0.34 a
64USA09	0.60 ±0.04 f	2.33 ±0.05 g	0.27 ±0.01 g	9.48 ±0.24 c
64USA10	0.64 ±0.04 f	7.02 ±0.14 a	0.83 ±0.75 a	9.80 ±0.25 c
64USA11	0.46 ±0.03 h	3.90 ±0.08 c	0.54 ±0.03 c	9.28 ±0.23 d
64USA12	0.72 ±0.05 e	2.88 ±0.06 f	0.38 ±0.02 f	8.63 ±0.22 e
64USA13	0.75 ±0.05 d	3.27 ±0.07 e	0.45 ±0.02 e	13.38 ±0.33 a

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

homogenization, sonication and magnetic stirring, determined gallic acid: 5.81, 4.32 and 3.57 mg 100 g^{-1}, protocatechuic acid: 3.49, 2.30 and 2.69 mg 100 g^{-1}, vanillic acid: 4.57, 3.95 and 3.70 mg 100 g^{-1}, chlorogenic acid: 20.47, 17.03 and 24.45 mg 100 g^{-1}, and syringic acid: 9.19, 6.31 and 8.48 mg 100 g^{-1}, respectively. It is believed that the most important reason for the differences in the results of this study and other studies are because of the genetic, climatic conditions.

Organic acids

Statistically significant differences ($p < 0.05$) emerged among the genotypes of black mulberry in terms of the concentration of organic acids (Tab. 4). Oxalic acid, citric acid, tartaric acid and malic acid were determined to be in the following ranges: 0.45-1.25, 2.12-7.02, 0.22-0.86 and 6.65-13.65 g 100 g^{-1}, respectively.

Özgen et al. (2009) and Sanchez et al. (2014) have reported that malic acid and citric acid are concentrated as the organic acids present in the mulberry fruit. Gundogdu et al. (2011) measured citric acid, tartaric acid and malic acid as 1.084, 0.123 and 1.323 g 100 g^{-1}, respectively, in the black mulberry genotypes. Eyduran et al. (2015) reported that the predominant organic acid in all the genotypes was malic acid and its level was between 1.13 and 3.04 g 100 g^{-1}. Geçer et al. (2016) determined the highest values of malic acid content among organic acids of black and white mulberry as 3.07 and 2.13 g 100 g^{-1}, respectively. In the

Table 5. Sugar content of mulberry genotypes (mean for 2016 and 2017 ±SE)

Genotypes	Glucose (g 100 g^{-1})	Fructose (g 100 g^{-1})	Sucrose (g 100 g^{-1})
64USA01	9.13 ±0.16 b*	6.54 ±0.12 e	1.51 ±0.03 c
64USA02	10.33 ±0.18 a	5.94 ±0.11 f	1.28 ±0.02 d
64USA03	9.16 ±0.16 b	5.77 ±0.10 g	1.23 ±0.02 de
64USA04	9.15 ±0.16 b	8.85 ±0.16 a	1.50 ±0.03 c
64USA05	8.05 ±0.14 d	7.69 ±0.14 c	1.28 ±0.02 d
64USA06	7.92 ±0.14 e	6.98 ±0.12 d	1.08 ±0.02 g
64USA07	7.76 ±0.14 f	7.96 ±0.14 b	1.21 ±0.02 ef
64USA08	7.18 ±0.13 g	7.60 ±0.14 c	1.16 ±0.02 f
64USA09	8.09 ±0.14 d	6.84 ±0.12 d	1.79 ±0.03 b
64USA10	6.99 ±0.12 h	6.57 ±0.12 e	2.14 ±0.04 a
64USA11	8.05 ±0.14 d	5.77 ±0.10 g	1.28 ±0.02 d
64USA12	8.62 ±0.15 c	5.68 ±0.10 g	1.20 ±0.02 ef
64USA13	9.06 ±0.16 b	6.98 ±0.12 d	1.48 ±0.03 c

*Difference between means designated with the same letter in the same column is not significant at 0.05 level

same study, citric acid, tartaric acid, succinic acid and fumaric acid contents were found to range from 0.48 to 1.03 g 100 g^{-1}, 0.15 to 0.43 g 100 g^{-1}, 0.12 to 0.44 g 100 g^{-1}, and 0.01 to 0.12 g 100 g^{-1}, respectively. Gundogdu et al. (2017) found caffeic acid (21.09-2.44 mg 100 g^{-1}), syringic acid (11.91 -1.16 mg 100 g^{-1}), and *p*-coumaric acid (5.67-0.70 mg 100 g^{-1}). In this study, it was determined that the genotypes 64USA01, 64USA07, 64USA08, 64USA10 showed desirable features when compared to other genotypes in terms of phenolic compounds. The differences between studies are thought to be related to cultural practices, genetic differences and climate.

Sugar content

In this study, the values of glucose, fructose and sucrose were estimated in the fruits of mulberry genotypes (Tab. 5). The level of glucose was determined between 6.99 and 10.33 g 100 g^{-1}. The highest level of fructose was 8.85 g 100 g^{-1} and the lowest was 5.68 g 100 g^{-1}. Sucrose was determined in the range 1.16-2.14-g 100 g^{-1}.

Özgen et al. (2009) reported that the glucose and fructose values of different mulberry genotypes ranged from 5.50 to 7.12 g 100 mL^{-1}, and 4.86 to 6.41 g 100 mL^{-1}, respectively. Sanchez et al. (2014) revealed that the predominant sugar was fructose (61%) followed by glucose (39%). Mikulic-Petkovsek et al. (2012) found that fructose and glucose were the predominant sugars in the fruits of different genotypes, and they revealed that glucose and fructose contents were 3.68 and 3.99 g 100 g^{-1}, respectively, for *Morus nigra* L. genotypes in Slovenia. It is thought that the most important reasons for the differences among studies are related to genetic differences and climate.

CONCLUSIONS

In this research, the biochemical characteristics of the fruits of black mulberry genotypes grown in the province of Uşak were investigated. The black mulberry genotypes showed valuable phytochemical properties and have potential to be used in food and healthcare industries. The results on the phenolic and phytochemical properties of the mulberry genotypes may be of use to both consumers and agricultural companies supply important genetic sources for breeding studies. The present genetic resources of our country are important for the development of new varieties and we hope that this research will provide a source for such studies. The genotypes 64USA08, 64USA06 and 64USA10 can be recommended, in terms of chemical content, as the most valuable for future investigations.

CONFLICT OF INTEREST

Author declares no conflict of interest.

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