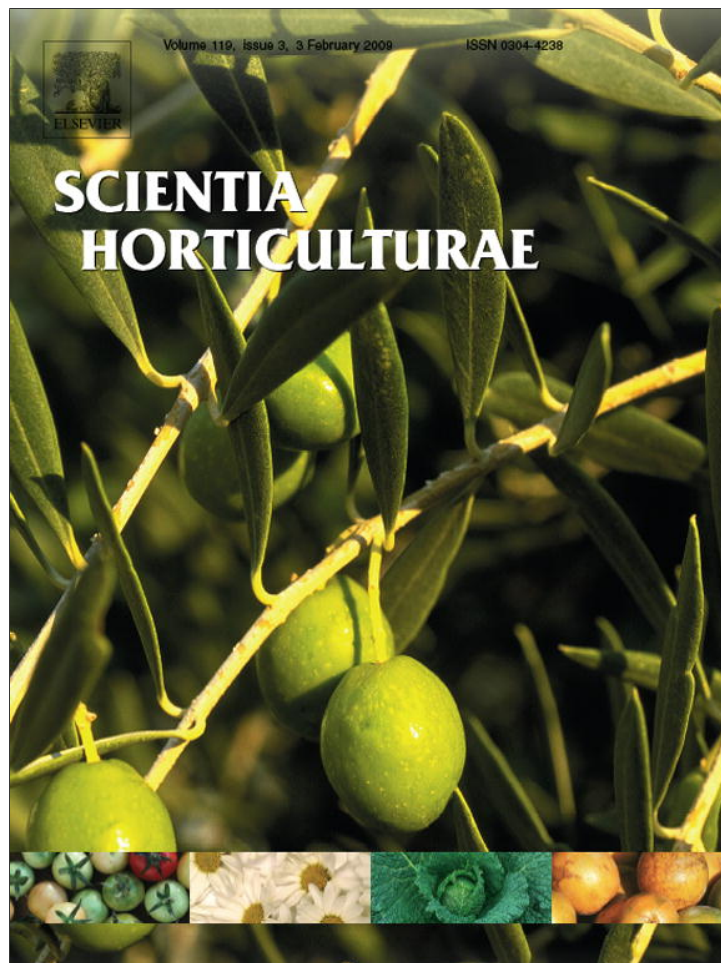


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Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *Morus rubra* fruits

Mustafa Özgen^{a,*}, Sedat Serçe^b, Cemal Kaya^c

^a Department of Horticulture, Faculty of Agriculture, University of Gaziosmanpaşa, 60240 Tokat, Turkey

^b Department of Horticulture, Faculty of Agriculture, Mustafa Kemal University, 31040 Antakya, Hatay, Turkey

^c Department of Food Engineering, Faculty of Agriculture, University of Gaziosmanpaşa, 60240 Tokat, Turkey

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ABSTRACT

In this study, phytochemical and antioxidant properties of anthocyanin-rich mulberry species of *Morus nigra* L. (black mulberry) and *Morus rubra* L. (red mulberry) fruits harvested from across Turkey were investigated. Fruit color, total phenolics (TP), total monomeric anthocyanin (TMA), titratable acidity (TA), and individual sugar and organic acid compositions were determined. Total antioxidant capacity (TAC) of fruits was assessed by both the trolox-equivalent antioxidant capacity (TEAC) and the ferric reducing antioxidant power (FRAP) assays. Black mulberry exhibited higher TP, TMA, TAC and TA when compared to red mulberry. The average TP contents of *M. nigra* and *M. rubra* were 2737 and 1603 µg gallic acid equivalent in g fresh weight basis (GAE/g fw), respectively. *M. nigra* had the richest amount of anthocyanin with an average of 571 µg cy-3-glu/g fw. Overall, TAC averaged 10.5 and 12.0 mmol TE/L by the TEAC and FRAP methods, respectively. We found that FRAP, TEAC, TP and TMA were significantly correlated ($r = 0.64\text{--}0.99$) with each other. Fructose (5.27 g/100 mL) and glucose (5.81 g/100 mL) were determined to be the major sugars in both mulberries. *M. nigra* displayed a higher TA (2.05 g/100 mL) than *M. rubra* (0.78 g/100 mL), with citric acid as the major acid.

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1. Introduction

Mulberry (*Morus* sp.) has been domesticated over thousands of years and has been adapted to a wide area of tropical, subtropical, and temperate zones of Asia, Europe, North and South America, and Africa. The most important widely grown anthocyanin-rich *Morus* species are *Morus alba*, *Morus rubra*, and *Morus nigra*. *Morus alba* has white and purple fruits with a very sweet taste and low acidity. Its fruits are perishable and mostly used for fresh consumption. *M. rubra*, known as “red mulberry”, is high in dry matter and has a sweet taste and low acidity. *M. nigra*, known as “black mulberry”, has juicy fruits with extraordinary color and a unique, slightly acidic flavor. Mulberry trees have historically been used for leaf yield in sericulture. In addition, their fruit, roots and bark have been used in folk medicine (especially in Chinese medicine) to treat diabetes, hypertension, and anemia and arthritis. Also, black mulberry fruits are used for treating mouth lesions in Turkey. Recently, red and black mulberries have gained an important position in the food industry due to the presence of anthocyanins.

Several researchers have previously reported that anthocyanins have remarkable antioxidant and free-radical scavenging activities (Stintzing et al., 2002; Wang et al., 1997). Additionally, multiple findings suggest that anthocyanin contents of berries and red fruits may provide possible health benefits such as reduced risk of coronary heart disease, stroke, certain types of cancers and aging (Prior, 2003; Zafra-Stone et al., 2007).

Identification and quantification of anthocyanins, phenolics and antioxidant properties of red fruits, especially berries, are well defined (Özgen et al., 2007; Sun et al., 2002; Çelik et al., 2008). Also, there are a number of detailed studies showing health benefits of the individual fruits. However, studies on characterization and quantification of phytochemical and antioxidant properties of mulberry fruits are very limited. Previously, Ercisli and Orhan (2007), Gerasopoulos and Stavroulakis (1997) and Güneş and Çekiç (2004) studied the quality and chemical characteristics of some of the *Morus* species; however, these studies were focused on specific local cultivars or genotypes with limited phytochemical and antioxidant properties. Lee et al. (2004) found that mulberries have cyanidin-based anthocyanins, particularly cyanidin-3-glucoside and cyanidin-3-rutinoside. However, the biological and pharmacological effects of these fruits are still poorly defined. In recent studies, Naderi et al. (2004) found that extracts of *M. nigra* fruits

* Corresponding author. Tel.: +90 356 2521616x2110; fax: +90 356 2521488.
E-mail address: mozgen@gop.edu.tr (M. Özgen).

have a protective action against peroxidative damage to biomembranes and biomolecules.

In this study, our objective was to investigate anthocyanin-rich mulberry species chosen from across Turkey as a nationwide selection program, and compare their phytochemical and antioxidant properties.

2. Materials and methods

2.1. Plant material

As a result of a nationwide mulberry selection program, anthocyanin-rich superior mulberry species of *M. nigra* (14 accessions) and *M. rubra* (four accessions) were sampled from across Turkey. Origin, location and genetic relationship of accessions were given in Kafkas et al. (2008). Fully mature fruits were harvested by hand and transferred to the laboratory for physical and phytochemical analysis. The fruit color was measured using a Minolta portable chromameter (Minolta, Model CR-400) which provided CIE L^* , a^* and b^* values. Samples were frozen immediately and then stored in about 100 g batches at $-30\text{ }^{\circ}\text{C}$ prior to analysis. For each fruit sample, three replicates were thawed at room temperature and homogenized in a standard food blender; excess fruits (40–60 individual fruits) were used to minimize naturally occurring fruit-to-fruit variation. Slurries were assayed for TA using standard methodology.

2.2. Analytical procedures

2.2.1. Determination of total phenolic (TP)

TP content was measured according to the Singleton and Rossi (1965) procedure. Briefly, fruit slurries were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5, v/v/v) for 1 h in darkness. Samples were replicated three times. Then, extracts were combined with Folin-Ciocalteu's phenol reagent and water, and incubated for 8 min followed by the addition of 7% sodium carbonate. After 2 h, the absorbance at 750 nm was measured by an automated UV–vis spectrophotometer (Model T60U, PG Instruments). Gallic acid was used as standard. The results were expressed as $\mu\text{g GAE/g fw}$.

2.2.2. Total monomeric anthocyanins (TMA)

TMA were estimated by a pH differential method (Giusti and Wrolstad, 2005) using a UV–vis spectrophotometer (Model T60U, PG Instruments). Absorbance was measured at 533 and 700 nm in buffers at pH 1.0 and 4.5 using $A = (A_{535} - A_{700})_{\text{pH}1.0} - (A_{535} - A_{700})_{\text{pH}4.5}$ with a molar extinction coefficient of 29,600. Results were expressed as $\mu\text{g cy-3-glu/g fw}$ basis.

2.2.3. The total antioxidant activity (TAC)

TAC was estimated by using two standard procedures, the FRAP and TEAC assays, as suggested by Özgen et al. (2006).

2.2.3.1. The ferric reducing ability of plasma (FRAP). FRAP was determined according to the method of Benzie and Strain (1996). The assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid, and 20 mmol/L ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1, v/v/v) to form the FRAP reagent just prior to analysis. For each assay, laboratory duplicates from each replicate plus 2.97 mL of FRAP reagent and 30 μL of sample extract were mixed. After 30 min the absorbance

of the reaction mixture at 593 nm was determined on a spectrophotometer.

2.2.3.2. Trolox equivalent antioxidant capacity (TEAC). For the standard TEAC assay, ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was dissolved in acetate buffer and prepared with potassium persulfate as described in Özgen et al. (2006). The mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability (Özgen et al., 2006). For the spectrophotometric assay, 2.97 mL of the ABTS⁺ solution and 30 μL of fruit extract were mixed and incubated for 10 min. The absorbance at 734 nm was then determined.

2.2.4. Extraction of individual sugars and organic acids

Mulberry slurries (5 g) were diluted with purified water or meta-phosphoric acid (2.5%) solution for individual sugar and organic acid analysis, respectively. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45 μm membrane filter (Iwaki Glass) before HPLC analysis, and the mobile-phase solvents were degassed before use. All the samples and standards were injected three times each, and mean values were used.

The HPLC analyses were conducted using a PerkinElmer HPLC system with Totalchrom navigator 6.2.1 software, a pump and UV detector (PerkinElmer, Series-200) (Waltham, Massachusetts, USA). Separation and determination of organic acids was done using a modified protocol from Shui and Leong (2002). The separation was performed with a SGE wakosil C18RS 5 μm column (250 mm \times 4.6 mm i.d.). Optimum efficiency of separation was obtained using a sulfuric acid solution with a pH of 2.5 (solvent A), and methanol (solvent B). Other parameters were adopted as follows: injection volume, 20 μL ; column temperature, 30 $^{\circ}\text{C}$; and detection wavelength, 215 nm.

Analysis of sugars was performed according to the method described by Bartolome et al. (1995) using a refractive index (RI) detector (PerkinElmer). The separation was carried out on a SGE SS Exsil amino column (250 mm \times 4.6 mm i.d.). The elution solvent used contained 80% acetonitrile and 20% deionized water. The column was operated at 30 $^{\circ}\text{C}$ with 0.9 mL/min flow rate. Sample injection volume was 20 μL . Three replicates were used.

2.3. Statistical analysis

Data were analyzed using SAS software and procedures (SAS, 2005). Means and standard deviations were calculated using the TABULATE procedure. Calculations of analysis of variance tables were not statistically valid as the fruits of the wild accessions were not sampled from a common environment with an experimental design. To evaluate variation between accessions for variables tests, coefficient of variations (C.V.) were calculated and expressed as percentages. Correlation coefficients and their levels of significance were calculated using the CORR procedure; separate analyses were conducted for each species.

3. Results and discussion

Among the mulberries investigated in this study, black mulberry had the greatest averages of TP, TMA and TAC (Table 1). TP content averaged 2485 $\mu\text{g GAE/g fw}$, and several accessions had contents higher than 3000 $\mu\text{g GAE/g fw}$ (N24, N35, N49, N60 and N68). On average, the *M. rubra* group had 42% less TP content than *M. nigra*. Similar patterns were observed for TMA. *M. nigra* had a mean of 571 $\mu\text{g/g fw}$. The range of TMA was 253–830 $\mu\text{g cy-3-glu/g fw}$. Moreover, *M. nigra* fruits had the highest

Table 1

Total phenolic content (TP), anthocyanin (TMA), antioxidant capacity (TEAC and FRAP) and titratable acidity (TA) of mulberry fruits sampled from Turkey

Accession	TP ($\mu\text{g GAE/g fw}$)	TMA ($\mu\text{g cy-3-glu/g fw}$)	TEAC ($\mu\text{mol TE/g fw}$)	FRAP ($\mu\text{mol TE/g fw}$)	TA (g/100 mL)
<i>Morus nigra</i> (black mulberry)					
N9	2530 \pm 55	584 \pm 29	13.1 \pm 0.4	14.0 \pm 0.7	2.11 \pm 0.01
N21	2397 \pm 92	522 \pm 17	10.9 \pm 0.2	12.2 \pm 0.3	2.04 \pm 0.02
N22	2824 \pm 34	641 \pm 33	12.2 \pm 0.3	11.1 \pm 0.1	2.25 \pm 0.02
N24	3135 \pm 117	701 \pm 52	13.0 \pm 0.4	16.9 \pm 0.4	2.41 \pm 0.00
N35	3121 \pm 140	815 \pm 11	14.4 \pm 0.2	16.1 \pm 2.4	2.88 \pm 0.02
N44	2597 \pm 124	398 \pm 16	10.0 \pm 0.4	12.3 \pm 0.6	1.83 \pm 0.11
N45	1908 \pm 63	253 \pm 28	6.9 \pm 0.4	7.3 \pm 0.4	2.24 \pm 0.01
N49	3488 \pm 133	689 \pm 18	13.1 \pm 0.2	14.6 \pm 0.6	1.59 \pm 0.00
N54	2727 \pm 126	356 \pm 9	6.8 \pm 0.5	10.8 \pm 0.3	1.74 \pm 0.00
N60	3160 \pm 102	480 \pm 39	11.4 \pm 0.4	13.2 \pm 0.3	2.13 \pm 0.01
N62	2683 \pm 72	674 \pm 37	13.0 \pm 0.3	14.7 \pm 0.3	2.04 \pm 0.01
N63	2963 \pm 119	739 \pm 103	13.6 \pm 0.4	16.4 \pm 0.5	1.62 \pm 0.11
N68	3013 \pm 67	830 \pm 34	12.3 \pm 0.3	11.7 \pm 0.4	2.10 \pm 0.03
N76	1766 \pm 100	308 \pm 22	8.7 \pm 0.9	9.9 \pm 0.5	2.10 \pm 0.03
Mean	2737 \pm 480	571 \pm 23	11.4 \pm 2.4	12.9 \pm 2.7	2.08 \pm 0.33
<i>Morus rubra</i> (red mulberry)					
R2	1586 \pm 102	120 \pm 7	7.3 \pm 0.3	7.7 \pm 1.2	1.04 \pm 0.00
R6	1433 \pm 38	72 \pm 4	6.7 \pm 0.6	7.6 \pm 0.2	0.36 \pm 0.00
R7	1005 \pm 87	3 \pm 1	5.1 \pm 1.6	3.7 \pm 0.2	0.70 \pm 0.02
R8	2388 \pm 87	200 \pm 5	7.1 \pm 0.2	6.7 \pm 1.1	1.01 \pm 0.01
Mean	1603 \pm 578	98.8 \pm 2	6.6 \pm 0.6	6.4 \pm 0.5	0.78 \pm 0.32
Overall					
Mean	2485	486	10.5	12.0	1.79
S.D.	671	250	2.9	3.6	0.62
C.V. (%)	27	51	28	30	35

The accessions belong to *M. nigra* (N) and *M. rubra* (R). Values represent mean \pm S.D. calculated from three replicates.

TAC, with an average of 11.4 and the range of 6.8–14.4 $\mu\text{mol TE/g fw}$ by the TEAC method. The mean of *M. rubra* was about half of *M. nigra* while *M. rubra* accessions had even less activity as determined by TEAC. Similar patterns were observed for FRAP and TA. *M. nigra* had higher averages than *M. rubra* for both FRAP and TA. Similar to our results, Bae and Suh (2007) found similar

amounts of TP, but higher anthocyanin contents in unspecified species of mulberries. Ercisli and Orhan (2008) found moderate antioxidant activity in selected black mulberries using a different antioxidant method.

We observed considerable variation between the mulberry species for most of the traits tested. The external fruit color characteristics are presented in Table 2. The L^* values of the *M. nigra* group ranged from 14.4 to 27.9 with an average of 18.4. *M. rubra* accessions were less variable as they ranged from 11 to 14.2. a^* and b^* values had even greater variability than L^* values when compared by their C.V. values (60 and 69% to 30%, respectively). The highest a^* and b^* values obtained from *M. nigra* fruits were 18.3

Table 2

Several fruit color characteristics of mulberry fruits sampled from Turkey

Accession	L^*	a^*	b^*	Chroma	Hue $^\circ$
<i>Morus nigra</i> (black mulberry)					
N9	16.1 \pm 1.5	17.2 \pm 0.7	4.4 \pm 2.0	17.8 \pm 2.7	14.3 \pm 0.4
N21	17.9 \pm 2.6	18.5 \pm 1.1	6.0 \pm 1.7	19.4 \pm 3.1	18.1 \pm 0.7
N22	22.9 \pm 1.1	25.1 \pm 0.2	8.2 \pm 1.2	26.4 \pm 0.9	18.0 \pm 0.6
N24	16.2 \pm 0.9	13.7 \pm 0.6	3.2 \pm 1.7	14.1 \pm 1.7	13.3 \pm 1.0
N35	14.4 \pm 1.9	18.0 \pm 0.5	4.6 \pm 1.5	18.6 \pm 1.1	14.5 \pm 0.7
N44	16.3 \pm 1.0	13.0 \pm 0.2	3.2 \pm 1.5	13.3 \pm 0.7	13.7 \pm 0.3
N45	19.4 \pm 2.3	17.6 \pm 0.4	4.7 \pm 1.6	18.2 \pm 1.5	14.8 \pm 0.5
N49	14.9 \pm 2.3	10.2 \pm 0.4	2.6 \pm 2.5	10.6 \pm 1.4	14.0 \pm 0.6
N54	18.5 \pm 2.0	14.4 \pm 0.5	4.8 \pm 2.1	15.1 \pm 1.1	18.5 \pm 0.7
N60	15.4 \pm 1.7	15.8 \pm 0.9	5.4 \pm 1.3	16.7 \pm 2.0	18.8 \pm 1.2
N62	17.1 \pm 2.6	16.0 \pm 0.4	4.8 \pm 2.2	16.7 \pm 2.9	16.6 \pm 1.5
N63	15.1 \pm 2.6	20.5 \pm 0.9	6.0 \pm 3.9	21.3 \pm 3.1	16.3 \pm 0.6
N68	27.9 \pm 1.5	31.0 \pm 1.1	11.6 \pm 2.0	33.1 \pm 2.6	20.6 \pm 0.4
N76	25.9 \pm 3.1	25.0 \pm 0.9	8.1 \pm 0.8	26.3 \pm 2.8	18.0 \pm 0.4
Mean	18.4 \pm 1.9	18.3 \pm 1.9	5.5 \pm 0.6	19.1 \pm 2.0	16.4 \pm 0.7
<i>Morus rubra</i> (red mulberry)					
R2	14.2 \pm 1.0	1.4 \pm 0.4	0.2 \pm 5.7	1.5 \pm 0.3	9.6 \pm 11.4
R6	13.3 \pm 1.8	1.7 \pm 0.3	-0.3 \pm 5.2	1.7 \pm 0.6	15.9 \pm 7.3
R7	11.1 \pm 5.3	5.5 \pm 0.1	0.2 \pm 4.9	5.5 \pm 4.6	1.9 \pm 1.6
R8	13.5 \pm 0.9	1.5 \pm 0.4	0.5 \pm 5.3	1.6 \pm 0.5	19.0 \pm 11.9
Mean	13.0 \pm 2.2	2.5 \pm 1.5	0.2 \pm 0.3	2.6 \pm 1.5	11.6 \pm 5.0
Overall					
Mean	17.9	14.2	4.5	15.1	35.5
S.D.	5.4	8.5	3.1	8.7	76.0
C.V. (%)	30	60	69	58	214

The accessions belong to *M. nigra* (N) and *M. rubra* (R). The values shown are mean \pm S.D. from 10 replications.

Table 3Correlation coefficients (r) of total phenolics (TP), total anthocyanin (TMA), antioxidant capacity (FRAP and TEAC), and titratable acidity (TA) as a maturity indicator

Variable	TMA ^a	TEAC ^b	FRAP ^c	TA
TP ^d	0.70**	0.64**	0.68**	-0.04
	0.99**	0.56	0.97**	0.53
TMA		0.89**	0.72**	0.20
		0.62	0.99**	0.61
TEAC			0.81**	0.23
			0.59	0.15
FRAP				0.08
				-0.99**

The first value represents r for *M. nigra* accessions while the second represents r for *M. rubra* accessions.

^a TMA were determined by the pH-differential method of Giusti and Wrolstad (2005). Values are expressed as $\mu\text{g cy-3-glu/g fw}$.

^b TEAC values were determined by the method of Özgen et al. (2006). Values are expressed as $\mu\text{mol TE/g fw}$.

^c FRAP values were determined by the method of Benzie and Strain (1996). Values are expressed as $\mu\text{mol of TE/g fw}$.

^d TP contents were estimated by the Folin-Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as $\mu\text{g GAE/g fw}$.

** Significance at 5%.

Table 4
Mean individual sugar and organic acid contents (g/100 mL) of mulberry fruits sampled from Turkey

Accession	Sugar				Organic acid			
	Fructose	Glucose	Sucrose	Total	Malic acid	Ascorbic acid	Citric acid	Total
<i>Morus nigra</i> (black mulberry)								
N9	5.85 ± 0.06	7.03 ± 0.01	0.01 ± 0.00	12.9 ± 0.1	0.15 ± 0.01	0.004 ± 0.00	1.96 ± 0.00	2.11 ± 0.01
N21	6.06 ± 0.01	6.73 ± 0.04	0.05 ± 0.01	12.8 ± 0.5	0.15 ± 0.02	0.003 ± 0.00	1.88 ± 0.00	2.04 ± 0.02
N22	6.40 ± 0.09	7.12 ± 0.05	0.05 ± 0.01	13.6 ± 0.1	0.17 ± 0.02	0.004 ± 0.00	2.08 ± 0.00	2.25 ± 0.02
N24	6.14 ± 0.03	6.63 ± 0.02	0.04 ± 0.00	12.8 ± 0.5	0.16 ± 0.00	0.003 ± 0.00	2.25 ± 0.00	2.41 ± 0.00
N35	5.58 ± 0.02	6.15 ± 0.02	0.01 ± 0.00	11.7 ± 0.1	0.19 ± 0.01	0.005 ± 0.00	2.68 ± 0.00	2.88 ± 0.01
N44	6.41 ± 0.17	6.62 ± 0.12	0.03 ± 0.01	13.1 ± 0.2	0.23 ± 0.02	0.008 ± 0.00	1.60 ± 0.09	1.83 ± 0.11
N45	5.22 ± 0.03	5.62 ± 0.03	0.03 ± 0.00	10.9 ± 0.1	0.21 ± 0.02	0.010 ± 0.00	1.77 ± 0.00	1.99 ± 0.06
N49	5.80 ± 0.04	6.56 ± 0.08	0.07 ± 0.03	12.4 ± 0.1	0.14 ± 0.00	0.007 ± 0.00	1.45 ± 0.00	1.59 ± 0.00
N54	5.12 ± 0.11	5.77 ± 0.11	0.02 ± 0.01	10.9 ± 0.1	0.11 ± 0.00	0.006 ± 0.00	1.63 ± 0.00	1.74 ± 0.00
N60	4.86 ± 0.02	5.50 ± 0.04	0.04 ± 0.02	10.4 ± 0.1	0.10 ± 0.00	0.004 ± 0.00	2.03 ± 0.00	2.13 ± 0.01
N62	6.26 ± 0.04	6.96 ± 0.01	0.01 ± 0.00	13.2 ± 0.1	0.17 ± 0.00	0.004 ± 0.00	1.87 ± 0.00	2.04 ± 0.01
N63	–	–	–	–	0.14 ± 0.01	0.006 ± 0.00	1.48 ± 0.09	1.62 ± 0.11
N68	5.22 ± 0.03	5.87 ± 0.08	0.02 ± 0.01	11.1 ± 0.1	0.16 ± 0.01	0.004 ± 0.00	1.93 ± 0.02	2.10 ± 0.03
N76	5.56 ± 0.30	5.89 ± 0.28	0.03 ± 0.00	11.5 ± 0.5	0.16 ± 0.01	0.004 ± 0.00	1.93 ± 0.02	2.10 ± 0.03
Mean	5.77 ± 0.51	6.39 ± 0.57	0.03 ± 0.02	12.2 ± 1.1	0.16 ± 0.04	0.005 ± 0.00	1.87 ± 0.33	2.05 ± 0.33
<i>Morus rubra</i> (red mulberry)								
R2	4.43 ± 0.05	4.96 ± 0.03	0.10 ± 0.06	9.5 ± 0.1	0.13 ± 0.03	0.003 ± 0.00	0.91 ± 0.00	1.04 ± 0.03
R6	2.77 ± 0.06	2.85 ± 0.03	0.05 ± 0.00	5.7 ± 0.1	0.13 ± 0.00	0.004 ± 0.00	0.23 ± 0.00	0.36 ± 0.00
R7	4.66 ± 0.06	4.84 ± 0.06	0.04 ± 0.01	9.5 ± 0.1	0.30 ± 0.02	0.004 ± 0.00	0.39 ± 0.00	0.70 ± 0.02
R8	3.25 ± 0.04	3.63 ± 0.05	0.05 ± 0.02	6.9 ± 0.1	0.17 ± 0.02	0.005 ± 0.00	0.83 ± 0.00	1.01 ± 0.02
Mean	3.78 ± 0.91	4.07 ± 1.01	0.06 ± 0.03	7.9 ± 1.9	0.18 ± 0.08	0.004 ± 0.00	0.59 ± 0.33	0.78 ± 0.32
Overall								
Mean	5.27	5.81	0.04	11.12	0.16	0.00	1.60	1.76
S.D.	1.02	1.17	0.03	2.17	0.05	0.00	0.65	0.64
C.V. (%)	19	20	73	20	29	27	40	36

The accessions belong to *M. nigra* (N) and *M. rubra* (R). Values represent mean ± S.D. calculated from three replicates.

and 5.5, respectively, while N68 had the highest a^* and b^* among the black mulberries. High a^* and b^* values were indicated by their dark red color. These average values were smaller in *M. rubra* (2.5 and 0.2). A similar pattern was observed with C^* values and hue°.

Earlier studies of small fruit phytonutrient contents reported high correlations among TP, TMA and TAC as determined by the TEAC and FRAP assays (Özgen et al., 2007, 2008). In our study, the correlation coefficient, r , among TP, TMA, TEAC and FRAP was also high, and all pair-wise combinations were found to be significant at 5% for *M. nigra* accessions (Table 3). However, none of the other variables were significantly correlated to TA for *M. nigra*. Conversely, for *M. rubra* accessions, TA was found to be highly correlated to FRAP. The correlations of TEAC to TP, TEAC and FRAP were not significant. This is probably caused by the relatively low number of accessions for this species.

Individual sugars and organic acid contents of mulberries are presented in Table 4. Sugar compositions of the accessions were similar regardless of the species. The main sugar was glucose (about 52%) followed by fructose (about 48%). Sucrose was detected in some accessions; however, it only reached 1% of total sugars in a few accessions. The average sugar contents of *M. nigra* and *M. rubra* were 12.2 and 7.9%, respectively. The main organic acids for the mulberries were citric and malic acids. Tartaric (not shown in the table) and ascorbic acid were detected in trace amounts, where the highest amount obtained was from N45 at about 0.01 g/100 mL. Both the amounts and compositions of the organic acids were found to be variable for the species tested.

The organic acid compositions were similar within *M. nigra* and *M. rubra* accessions, but different between the species. For *M. nigra* accessions, citric acid was 92% of the total acid while malic acid was only 8%. For *M. rubra* accessions, citric and malic acids made up 72 and 27% of the total acids, respectively. The same ratios were 15 and 85%, respectively, for *M. laevigata*. TA was 2.05 and 0.78 for *M. nigra* and *M. rubra*, respectively.

4. Conclusion

In this study, we compared and characterized the phytonutrient content and antioxidant capacity of selected anthocyanin-rich mulberries, and determined the strength of the relationships among commonly measured variables. Red and black mulberry species displayed different characteristics. In addition to high antioxidant capacity black mulberry had high anthocyanin and phenolic contents, which may increase its popularity among the other mulberries or even among other fruits in general. These results, demonstrating superior horticultural and phytonutrient traits of mulberries, may also provide a basis for planning breeding strategies as well as selecting cultivars with high phytonutrient profiles and antioxidant capacities as functional foods for consumers. However, more detailed biological and pharmacological studies are still needed for additional clarification and better understanding of the health benefits of anthocyanin-rich mulberries.

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References

- Bae, S.H., Suh, H.J., 2007. Antioxidant activities of five different mulberry cultivars in Korea. *Lwt-Food Sci. Technol.* 40, 955–962.
- Bartolome, A.P., Ruperez, P., Fuster, C., 1995. Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of Red Spanish and Smooth Cayenne cultivars. *Food Chem.* 53, 75–79.

- Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239, 70–76.
- Çelik, H., Özgen, M., Serçe, S., Kaya, C., 2008. Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit. *Sci. Hortic.* 117, 345–348.
- Ercisli, S., Orhan, E., 2007. Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits. *Food Chem.* 103, 1380–1384.
- Ercisli, S., Orhan, E., 2008. Some physico-chemical characteristics of black mulberry (*Morus nigra* L.) genotypes from Northeast Anatolia region of Turkey. *Sci. Hortic.* 116, 41–46.
- Gerasopoulos, D., Stavroulakis, G., 1997. Quality characteristics of four mulberry (*Morus* sp.) cultivars in the area of Chania, Greece. *J. Sci. Food Agric.* 73, 261–264.
- Giusti, M.M., Wrolstad, R.E., 2005. Characterization and measurement of anthocyanins by UV–visible spectroscopy Unit F1.2. In: Wrolstad, R.E., Schwartz, S.J. (Eds.), *Handbook of Food Analytical Chemistry*. Wiley, New York, pp. 19–31.
- Güneş, M., Çekiç, Ç., 2004. Some chemical and physical properties of fruits of different mulberry species commonly grown in Anatolia, Turkey. *Asian J. Chem.* 16, 1849–1855.
- Kafkas, S., Özgen, M., Doğan, Y., Özcan, B., Ercişli, S., Serçe, S., 2008. Molecular characterization of mulberry accessions in Turkey by AFLP Markers. *J. Am. Soc. Hortic. Sci.* 133, 593–597.
- Lee, J.Y., Moon, S.O., Kwon, Y.J., Rhee, S.J., Park, H.R., Choi, S.W., 2004. Identification and quantification of anthocyanins and flavonoids in mulberry (*Morus* sp.) cultivars. *Food Sci. Biotechnol.* 13, 176–184.
- Naderi, G.A., Asgary, S., Sarraf-Zadegan, N., Oroojy, H., Afshin-Nia, F., 2004. Antioxidant activity of three extracts of *Morus nigra*. *Phytother. Res.* 18, 365–369.
- Özgen, M., Reese, R.N., Tulio, A.Z., Miller, A.R., Scheerens, J.C., 2006. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *J. Agric. Food Chem.* 54, 1151–1157.
- Özgen, M., Serçe, S., Gunduz, K., Yen, F., Kafkas, E., Paydas, S., 2007. Determining total phenolics and antioxidant capacities of selected *Fragaria* genotype. *Asian J. Chem.* 19, 5573–5581.
- Özgen, M., Durgaç, C., Serçe, S., Kaya, C., 2008. Chemical and antioxidant properties of pomegranate cultivars grown in mediterranean region of Turkey. *Food Chem.* 111, 703–706.
- Prior, R.L., 2003. Absorption and metabolism of anthocyanins: potential health effects. In: *Phytochemicals: Mechanisms of Action*. CRC Press Inc., Boca Raton, FL.
- SAS Institute, 2005. SAS Online Doc, Version 8. SAS Inst., Cary, NC.
- Shui, G., Leong, L.P., 2002. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. *J. Chromatogr. A* 977, 89–96.
- Singleton, V.L., Rossi, J.L., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Viticult.* 16, 144–158.
- Stintzing, F.C., Carle, R., Frei, B., Wrolstad, R.E., 2002. Color and antioxidant properties of cyanidin-based anthocyanin pigments. *J. Agric. Food Chem.* 50, 6172–6180.
- Sun, J., Chu, Y.F., Wu, X., Liu, R.H., 2002. Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* 50, 7449–7454.
- Wang, H., Cao, G., Prior, R.L., 1997. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* 45, 304–309.
- Zafra-Stone, S., Yasmin, T., Bagchi, M., Chatterjee, A., Vinson, J.A., Bagchi, D., 2007. Berry anthocyanins as novel antioxidants in human health and disease prevention. *Mol. Nutr. Food Res.* 51, 675–683.