

Organic Acid Composition of Selected Mulberry Genotypes from Aras Valley

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ABSTRACT This study was carried out to determine the organic acid content of selected 3 white (*Morus alba* L.) and 3 black mulberry (*Morus nigra* L.) genotypes grown in Aras Valley of Turkey between 2011 and 2013 years. High Performance Liquid Chromatography (HPLC) system was used for detecting organic acids. Organic acid contents of white and black mulberry genotypes showed very significant differences in according to year and genotype. Fluctuation detected over the years. Higher organic acid concentrations were found in Genotype 8 (G8) in white mulberries and Genotype 3 (G3) in black mulberries than the other genotypes. Citric acid content of black mulberries were found approximately 5 times higher than white mulberries in general. For the other organic acids white mulberries had higher concentrations than the black mulberries. The average organic acid concentrations of white and black mulberry fruits were determined as 1.805-8.855 mg g⁻¹ for citric acid, 0.145-0.115 mg g⁻¹ for tartaric acid, 9.095-5.215 mg g⁻¹ for malic acid, 2.836-0.755 mg g⁻¹ for succinic acid, 0.662-0.329 mg g⁻¹ for lactic acid, 0.213-0.015 mg g⁻¹ for fumaric acid and 0.053-0.054 mg g⁻¹ for acetic acid, respectively.

Keywords: Organic acids, *Morus alba* L., *Morus nigra* L., Aras Valley

Aras Vadisi'nden Selekte Edilen Dut Genotiplerinin Organik Asit Kompozisyonu

ÖZET : Bu araştırma daha önceki çalışmalarda Aras Vadisi'nden seçilen 3 beyaz (*Morus alba* L.) ve 3 kara (*Morus nigra* L.) dut genotipinin organik asit seviyelerini tespit etmek amacıyla 2011-2013 yılları arasında yürütülmüştür. Organik asitlerin belirlenmesinde Yüksek Performanslı Sıvı Kromatografisi (HPLC) sistemi kullanılmıştır. Beyaz ve karadut genotiplerinin organik asit içerikleri yıllara ve genotiplere göre çok önemli oranda farklılık göstermiştir. Yıllara göre düzensiz değişim söz konusu iken, beyaz dut genotiplerinden Genotip 8, karadut genotiplerinden Genotip 3'ün organik asit içerikleri bakımından daha yüksek değerlere sahip olduğu tespit edilmiştir. Genel olarak karadutların sitrik asit içeriği, beyaz dutların içeriğinden yaklaşık 5 kat daha fazla bulunmuştur. Diğer organik asit içerikleri açısından beyaz dutların daha zengin olduğu belirlenmiştir. Beyaz ve karadutların ortalama sitrik, tartarik, malik, sukkinik, laktik, fumarik ve asetik asit içeriği sırasıyla 1,805-8,855 mg g⁻¹; 0,145-0,115 mg g⁻¹; 9,095-5,215 mg g⁻¹; 2,836-0,755 mg g⁻¹; 0,662-0,329 mg g⁻¹; 0,213-0,015 mg g⁻¹ ve 0,213-0,015 mg g⁻¹ değerleri arasında bulunmuştur.

Anahtar kelimeler: Organik Asitler, *Morus alba* L., *Morus nigra* L., Aras Havzası

INTRODUCTION

Mulberry (*Morus* spp.) is cultivating for consuming its fruits as fresh, dried or processed (Orhan et al. 2007) as well as using in sericulture and animal husbandry (Vijayan, 2009). Most of the mulberry cultivars are believed to have originated in the Himalayan foothills and later dispersed into Asia, Europe, Africa and America and has been cultivated in the Northern hemisphere and Turkey for centuries (Ipek et al. 2012; Karlıdag et al. 2012). Mulberry production was 69.334 ton in 2015, is as important as the other temperate fruit species in Turkey (Anonymous, 2015).

Mulberry fruits are rich in organic acids, phenolics, anthocyanins and minerals (Koyuncu, 2004; Ercisli and Orhan, 2007; Gundogdu et al. 2012), which makes it valuable health and dietary product (Kamiloglu et al. 2013). Consumption of berries has potential health effects against cancer, anti-aging and neurological diseases, inflammation, diabetes, and bacterial infections (Takasaki et al. 1982; Hikino et al. 1985; Baytop, 1996; Lee et al. 1998; Kim et al. 1999; Kusano et al. 2002). Acidity that plays an important role in the perception of fruit

quality, affects not only the sour taste of the fruit, but also sweetness, by masking the taste of sugars (Lobit et al. 2006). The proportions of individual acids are also important. Citric acid masked the perception of sucrose and fructose (Schifferstein and Fritjers, 1990, Bonnans and Noble, 1993) while malic acid enhanced sucrose perception. The nature and concentration of organic acids are important factors influencing the organoleptic properties and can maintain the nutritive value of fruit (Daood et al. 1994; Silva et al. 2002). They are also used extensively as additives, namely antioxidant, acidulants, or preservatives (Koyuncu, 2004; Cemeroglu et al. 2004). Acids found in fruits have no negative effects on human body, as they are rapidly oxidized during the metabolism (Schobinger, 1988). Some of them are citric, malic and tartaric acid and are predominant in most fruit species (Cemeroglu et al. 2004).

Organic acids also play an important role in plant physiology as cofactors, buffering agents, and intermediates of the most important metabolic pathways of carbohydrates, lipids, proteins and

sources of respiratory energy in fruits (Koyuncu, 2004; Cemeroglu et al. 2004) and their content significantly affected by temperature and rainfall (De La Hera Orts et al. 2005; Sweetman et al. 2014).

Recently, there have been some studies on organic acids content of mulberry fruit (Koyuncu, 2004; Ercisli and Orhan, 2007; Ozgen et al. 2009; Gundogdu et al. 2011; Shengfeng et al. 2011). However, to our knowledge, there are no data available the effects of year factor on individual organic acids in mulberries. Monthly climatic properties (especially temperature and rainfall) can change notably year by year. Therefore, the objective of this work was to study the impact of climatic conditions over three years on individual organic acid concentration in selected mulberry genotypes from

Morus alba and *Morus nigra* and determine differences among genotypes belonging to each species and between species.

MATERIAL AND METHOD

Climatic conditions

The three years of study differed highly in climatic conditions. Average temperature, total rainfall, and humidity from January to harvest date of June are showed Table 1. In 2011, the average temperature in April, May and June was lower, and the total rainfall and the average humidity from February to June were higher than in the other two years. The study area is located 39.57° N latitude, 43.40° E longitude and about 880 meters altitude (above sea level).

Table 1. Monthly acquired meteorological data from January to June for Aras Valley in the year 2011, 2012 and 2013.

Month/Years	Temperature °C			Rainfall (mm)			Humidity (%)		
	2011	2012	2013	2011	2012	2013	2011	2012	2013
January	-6.0	0.3	-2.2	6.0	0.0	19.6	74.0	59.5	71.9
February	0.7	-3.1	4.3	22.6	12.5	15.2	66.1	63.0	64.3
March	7.8	3.6	9.2	16.8	13.5	14.8	48.5	47.1	44.5
April	13.5	16.1	17.7	73.9	16.2	34.6	57.7	43.1	40.0
May	17.5	19.7	20.1	76.9	57.4	58.9	59.8	51.3	38.2
June	23.5	25.0	24.7	40.4	26.7	38.3	47.0	37.6	44.8
Average	9.5	10.3	12.3	-	-	-	58.9	50.3	50.6
Total	-	-	-	236.6	126.3	181.4	-	-	-

Plant Materials

Fruit samples of three genotypes that were Genotype-6 (G6), Genotype-8 (G8) and Genotype-9 (G9) of white mulberry and three genotypes that were Genotype-2 (G2), Genotype-3 (G3) and Genotype-5 (G5) of black mulberry were selected from white and black mulberry populations according to some physical fruit characteristics such as fruit weight and size and harvested at commercially maturity stage on the June of 2011, 2012 and 2013 from Aras Valley of Turkey. Mulberry trees used in study were close to each other in terms of age and growing status. About 1 kg samples for each genotype were taken and divided into three groups for three replicates and kept in plastic bags at -25 °C until analyses.

Source of Chemicals

All acids and reagents were of analytical grade. Analytical standards that were malic, tartaric, citric, lactic, succinic, fumaric and acetic acid were purchased from Sigma-Aldrich (St. Louis, MO). Ultra distilled water was used in the preparation of

fruit samples, the standard solution and the mobile phase.

Apparatus

HPLC analysis of organic acids was performed on a Shimadzu Prominence-series HPLC system equipped with a Liquid chromatograph (LC-20AT), degasser (DGU-20A₅), auto-sampler (SIL-20AHT), column oven (CTO-20A) and diode array detector (DAD) (SPD-M20A).

Extraction of organic acids

The method of organic acid extraction by Gundogdu et al. (2011) was carried out by a minor modification. In this modification, 0.005 N H₂SO₄ was used instead of 0.009 N H₂SO₄. About 200 g samples from each genotype were fragmented and 5 g was transferred into centrifuge tubes. The samples were supplemented with a 10 ml of 0.005 N H₂SO₄ and were homogenized with a WiseTis HG-15D (Daihan Scientific Co., Korea). After that, the samples were mixed for an hour with a IKA KS 4000i (German) shaker and centrifuged at 15,000 x g

for 15 min with an Universal 320 R (Hettich, German) centrifuge. The supernatant were passed through coarse filter paper and a 0.45 μm membrane filter (Millipore Corp., Bedford, MA, USA) under vacuum two times, and last in the SEP-PAK C18 cartridge. Organic acids extracted samples stored in a refrigerator and analyzed within 12 h of preparation (Mahmood et al. 2012).

HPLC Conditions and Detection of Organic Acids

For organic acid determination, the method by Bevilacqua and Califano (1989) was modified. Organic acid separation was carried out using an Agilent Hi-Plex H ($8\mu\text{m}$, 300mm x 7.7 mm i.d.) column (Agilent Technologies, USA). An isocratic mobile phase consisted of 0.005 N H_2SO_4 and was delivered at a flow rate of 0.6 mL min^{-1} . Column oven temperature was adjusted 55 $^\circ\text{C}$. The mobile phase was filtered through membrane filter (47 mm, 0.45 μm) and was sonicated for 10 min in an ultrasonic bath (Wise Clean-WUC-A03H) to remove

air bubbles before use. The injection volume was 20 μL and target compounds were detected at 210 nm. Chromatographic data was collected and processed using LC Solution software (Shimadzu, Japan). Identification of organic acids was based on retention time and UV spectrum (Figure 1). A four point calibration curve was established for each standard compound. Therefore, the concentration of the mixture solution was injected into the HPLC-DAD system as follow; between 0.003 g L^{-1} - 3 g L^{-1} for citric acid, 0.0012 g L^{-1} - 1.2 g L^{-1} for tartaric acid, 0.001425 g L^{-1} - 1.425 g L^{-1} for malic acid, 0.0012 g L^{-1} - 1.2 g L^{-1} for succinic acid, 0.0003 g L^{-1} - 0.3 g L^{-1} for lactic acid, 0.00012 g L^{-1} - 0.12 g L^{-1} for fumaric acid and 0.0004 g L^{-1} - 0.4 g L^{-1} for acetic acid. All the calibration curves for organic acids displayed a good linear relationship with correlation coefficients above 0.999. The concentrations of the compounds were calculated from peak area according to calibration curves. The concentrations of organic acids for samples are represented as mg g^{-1} fresh weight (fw).

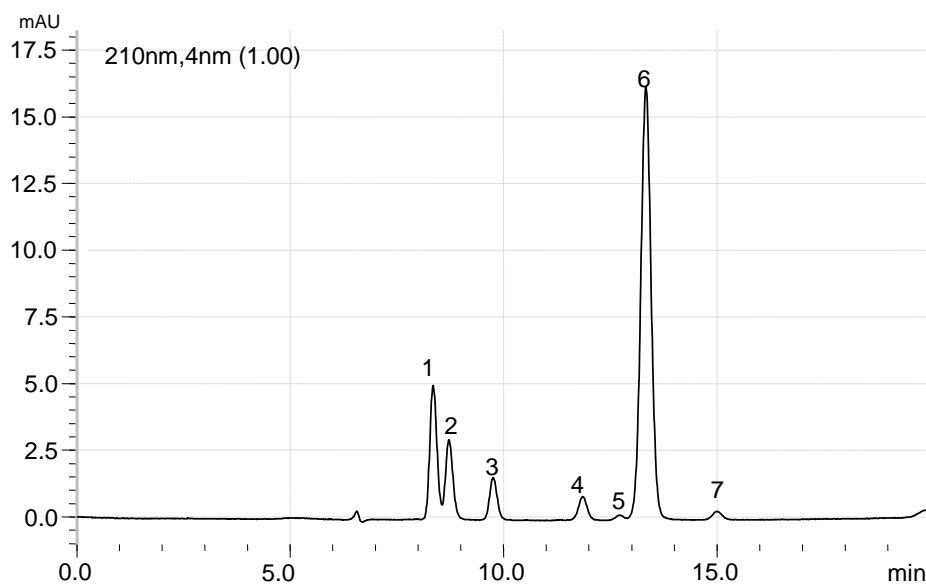


Figure.1 Chromatogram of organic acid mixture standart in Hi-Plex H column ($8\mu\text{m}$, 300mm x 7.7 mm i.d.). Peak identification; 1. Citric acid, 2. Tartaric acid, 3. Malic acid, 4. Succinic acid, 5. Lactic acid, 6. Fumaric acid, 7. Acetic acid.

Data analysis

Parameters were examined separately by ANOVA for three years of the study. Average separation by LSD test at $P < 0.01$ was used to determine whether there were significant differences among genotypes within each species in all three years. Two species were also compared to each other.

RESULTS AND DISCUSSION

The current study showed that among genotypes (G6, G8 and G9) belonging to *Morus alba* L. (white mulberry) and among genotypes (G2, G3 and G5) belonging to *Morus nigra* L. (black mulberry) were found significantly differences investigated the organic acids content of mulberry fruit in the three study years (Table 2). As seen from these results, on average of white mulberry

genotypes, G8 had more citric, malic, succinic, lactic and acetic acid concentration than the other two white mulberry genotypes. On average of black mulberry genotypes, G3 was found to have more tartaric, malic, succinic, fumaric and acetic acid concentration than the other black mulberry genotypes. Significant differences were also found between mulberry species in terms of all organic

acids content of mulberry fruit except acetic acid. When species compared to each other, white mulberry had more tartaric, malic, succinic, lactic and fumaric acid concentration than black mulberry with 0.145 mg g⁻¹, 9.095 mg g⁻¹, 2.836 mg g⁻¹, 0.662 mg g⁻¹ and 0.213 mg g⁻¹ values, respectively. However, black mulberries contained more citric acid than white mulberry with 8.855 mg g⁻¹ value.

Table 2. Organic acid concentration of white and black mulberry genotypes (mg g⁻¹ fw)

Species	Years	Genotypes	Citric acid	Tartaric acid	Malic acid	Succinic acid	Lactic acid	Fumaric acid	Acetic acid	
White Mulberry (<i>Morus alba</i> L.)	2011	G6	0.257 c	0.199 b	9.972 b	2.103 b	0.831 b	0.312 b	0.042 c	
		G8	1.675 b	0.212 a	7.900 c	5.665 a	1.675 a	0.389 a	0.063 b	
		G9	2.752 a	0.188 c	10.906 a	2.864 b	0.490 c	0.384 a	0.073 a	
	2012	G6	1.929 b	0.102 b	8.139 b	1.735 b	0.539 b	0.193 a	0.053 b	
		G8	3.850 a	0.111 a	9.273 a	2.606 a	0.686 a	0.086 b	0.096 a	
		G9	1.612 b	0.085 c	8.032 b	1.021 c	0.266 c	0.186 a	0.044 b	
	2013	G6	0.634 b	0.164 a	7.368 b	3.048 b	0.347 b	0.151 a	0.029 b	
		G8	2.956 a	0.130 b	12.492 a	4.085 a	0.829 a	0.058 b	0.047 a	
		G9	0.578 b	0.111 c	7.775 b	2.396 c	0.290 b	0.155 a	0.029 b	
	Genotypes average									
			G6	0.940 c	0.155 a	8.493 c	2.295 b	0.572 b	0.219 b	0.042 b
			G8	2.827 a	0.151 b	9.888 a	4.119 a	1.063 a	0.177 c	0.069 a
			G9	1.648 b	0.128 b	8.904 b	2.094 c	0.349 c	0.242 a	0.049 b
	Years average									
		2011		1.562 b	0.200 a	9.593 a	3.544 a	0.999 a	0.361 a	0.059 a
	2012		2.464 a	0.099 c	8.481 c	1.787 c	0.497 b	0.155 b	0.065 a	
	2013		1.389 c	0.135 b	9.212 b	3.176 b	0.489 b	0.121 c	0.035 b	
Black Mulberry (<i>Morus nigra</i> L.)	2011	G2	8.363 a	0.093 c	7.313 a	1.115 a	0.332 b	0.013 b	0.037 a	
		G3	6.781 b	0.137 b	7.361 a	1.081 b	0.308 c	0.010 c	0.039 a	
		G5	5.885 c	0.180 a	5.119 b	0.948 c	0.373 a	0.017 a	0.012 b	
	2012	G2	12.017 a	0.108 b	3.851 b	0.290 b	0.311 a	0.010 b	0.039 b	
		G3	12.077 a	0.136 a	4.845 a	0.358 a	0.271 b	0.025 a	0.100 a	
		G5	6.801 b	0.061 c	3.529 b	0.166 c	0.299 a	0.013 b	0.047 b	
	2013	G2	6.508 b	0.106 b	4.696 b	1.061 a	0.307 b	0.010 b	0.079 b	
		G3	7.739 b	0.114 a	5.168 a	1.052 a	0.304 b	0.025 a	0.092 a	
		G5	13.525 a	0.101 c	5.050 a	0.725 b	0.454 a	0.013 b	0.052 c	
	Genotypes average									
			G2	^{NS} 8.963	0.102 c	5.287 b	0.822 a	0.317 b	0.011 c	0.049 b
			G3	8.866	0.129 a	5.791 a	0.830 a	0.294 b	0.020 a	0.077 a
			G5	8.737	0.114 b	4.566 c	0.613 b	0.376 a	0.014 b	0.037 c
	Years average									
		2011		7.010 c	0.136 a	6.598 a	1.048 a	0.338 a	0.013 b	0.029 c
	2012		10.298 a	0.101 c	4.075 c	0.271 c	0.294 b	0.016 a	0.059 b	
	2013		9.257 b	0.107 b	4.971 b	0.946 b	0.355 a	0.016 a	0.074 a	
Species average										
		<i>M. alba</i> L.	1.805 b	0.145 a	9.095 a	2.836 a	0.662 a	0.213 a	^{NS} 0.053	
		<i>M. nigra</i> L.	8.855 a	0.115 b	5.215 b	0.755 b	0.329 b	0.015 b	0.054	

Values with different letters in a line are not significantly different at the 0.01 level according to LSD test

NS; not-significant

Koyuncu (2004) have reported that many fold higher concentration of malic and tartaric acid and 1.5-1.3 fold higher concentration of citric and fumaric acid respectively in 14 native black mulberry genotypes compared to results obtained in the present study. Ozgen et al. (2009) stated that black mulberry contained higher citric acid than the other organic acids. Similarly, Shengfeng et al. (2011) found that the most predominant organic acid was citric acid followed by malic acid and succinic acid in 33 black mulberry cultivars. Our results are consistent with those of detected in Turkey (Ozgen et al. 2009) and those of detected in China (Shengfen et al. 2011) in terms of citric acid dominance in black mulberry. Gundogdu et al. (2011) have reported the concentration of organic acids in white and black mulberry fruit as follows; 0.393 g - 1.084 g 100g⁻¹ for citric, 0.223 g - 0.123 g 100g⁻¹ for tartaric, 3.095 g - 1.323 g 100g⁻¹ for malic, 0.168 g - 0.342 100g⁻¹ for succinic, 0.074 g - 0.049 g 100g⁻¹ for lactic, 0.024 g - 0.011 g 100g⁻¹ for fumaric and 0.008 g - 0.019 g 100g⁻¹ for acetic acid, and these results are compatible with the present study in terms of some individual organic acids. However, there are some differences when the present study compared to those of detected by Koyuncu (2004). These differences may be explained by ecological, agronomic and genetic factors.

According to climatic conditions of the years significantly affected white and black mulberry genotypes in terms of organic acids concentration. On average of years, both in white and in black mulberry, most of organic acid with its highest levels were obtained from the year 2011 (Table 2). In that year, there were cooler temperature and higher rainfall than the other two years (Table 1). This is in accordance with the fact that especially malic acid is dependent on extent of malate enzyme and malic dehydrogenase activities and appears to have a respiratory quotient that is temperature dependent (Preiner et al. 2013). Oliveira and Sousa (2009) reported that grapes grown in cooler climates had higher organic acids than those cultivated in warmer climates. Moreover, moderate irrigation or rainfall increased the organic acid concentrations of grapevine cultivars (Esteban et al. 1999; De La Hera Orts et al. 2005; Preiner et al. 2013). These results are consistent with the results obtained in the present study. Because climatic factors such as temperature and total rainfall discussed above may vary from year to year, depending on these factors, organic acid concentration of mulberry fruit may have changed among years in the current study.

Conclusions

As a result of three years study, climatic factor such as; total rainfall and average temperature might

affect organic acid content of black and white mulberries. The more humidity and the cooler temperatures led to the more accumulation of organic acids in the white and black mulberry genotypes. Organic acids from fruit and vegetables have been shown to play an important role in the prevention of chronic diseases (osteoporosis, obesity) and as a modulator of large intestinal function. In the current study, G8 belonging to white mulberry specie and G3 belonging to black mulberry specie have more organic acids than the other genotypes during three years study, so they may be more useful for human diet. These genotypes may be recommended to mulberry growers and breeders in terms of their high organic acid content capacity.

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