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RESEARCH ARTICLE

Effects of Different Harvest Dates on Some Fruit Quality Parameters and Health Promoting Compounds of *Morus alba* L. and *Morus nigra* L. Fruit

Mücahit Pehluvan*, Berna Doğru Çokran, M. Ramazan Bozhüyük

Iğdır University, Faculty of Agriculture, Department of Horticulture, Iğdır/Turkey

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ABSTRACT

Mulberries are important fruits in terms of health-promoting compounds such as phenolics, antioxidants and organic acids. However, limited studies have addressed the changes of health-promoting compounds as well as fruit quality parameters during harvest season which have a long duration about two months. This study was conducted to determine whether some bioactive compounds and fruit quality parameters may be changed significantly in this long harvest season. White and black mulberry fruits were collected at commercial maturity stage fifteen day intervals (from 1st July to 1st August) in three locations (Iğdır, Tuzluca and Kağızman) in Aras Valley. The content of some selected organic acids and phenolic compounds were analyzed in fresh fruit samples by HPCL/DAD. Antioxidant activity determined also on fresh fruit samples by Spectrophotometer. Fruit weight, width, length, fruit juice pH, titratable acidity (TA) and soluble solid content (SSC) were examined as some fruit quality parameters. Phenolic compounds and antioxidant activity in black mulberries were higher than in white mulberries at all harvest dates in three different locations. Phenolic compound levels and antioxidant activity increased towards the end of harvest season. Some fluctuations were observed organic acid levels during harvest season in Tuzluca and Kağızman locations while the acids increased towards the end of harvest season in Iğdır location. Fruit weight, width and length decreased while titratable acidity increased at the end of harvest season in three locations. On the other hand, some fluctuations were observed SSC and fruit juice pH during the harvest season. As a result of the study, contrary to get smaller fruit, content of its phenolic levels and antioxidant activity increased towards the end of harvest season. Late harvested fruits may be more beneficial for human health compared to earlier ones in the same harvesting season.

Faklı Hasat Tarihlerinin *Morus alba* L. and *Morus nigra* L Meyvelelerinin Bazı Kalite Parametreleri ve Sağlık İçin Faydalı Bileşikler Üzerine Etkileri

ÖZ

Dutlar fenolik bileşikler, antioksidantlar ve organik asitler gibi sağlık açısından faydalı bileşiklere sahip olmaları bakımından çok önemli meyvelerdir. Ancak dutlarda 2 ay kadar uzun süren hasat dönemi boyunca meyve kalitesi ve bu faydalı bileşiklerin değişimi ile ilgili çalışmalar sınırlıdır. Çalışmamız dutlarda bulunan bazı biyoaktif bileşikler ile meyve kalitesinin bu uzun hasat döneminde önemli ölçüde değişip değişmediğini belirlemek amacı ile yürütülmüştür. Bu amaç doğrultusunda beyaz ve karadutlar Aras Vadisinde bulunan 3 lokasyondan (Iğdır, Tuzluca ve Kağızman) ve 3 farklı hasat tarihinde 15 günlük aralıklar ile (1 Temmuzdan, 15 Temmuz, 1 Ağustos) olgun dönemde toplanmışlardır. Meyvelerin bazı organik asit ve fenolik bileşikleri HPLC/DAD sistemi ile analiz edilmiştir. Antioksidant kapasite ise spektrofotometrik yöntem ile belirlenmiştir. Meyvelerin ayrıca ağırlık, en, boy, meyve suyu pH sı, titedilebilir asitlik ve SÇKM (Suda Çözünen Kuru Madde) değerleri incelenmiştir. Fenolik bileşikler ve antioksidant aktivite tüm hasat zamanlarında ve her üç lokasyonda karadutlarda beyaz dutlardan daha yüksek seviyede tespit edilmiştir. Fenolik bileşik seviyesi ve antioksidant kapasite hasat peryodunun sonuna doğru artış göstermiştir. Tuzluca ve Kağızman lokasyonlarında bazı dalgalanmalar ile beraber bir artış yaşanırken, Iğdır lokasyonunda ise meyvelerdeki organik asit miktarı hasat sezonu sonuna doğru artmıştır. Her üç lokasyonda da hasat peryodu sonuna doğru meyve ağırlığı, eni ve uzunluğunda azalış, titre edilebilir asitlikte ise artış meydana gelmiştir. Öte yandan SÇKM ve meyve suyu pH sın da da hasat peryodu boyunca dalgalanmalar gözlemlenmiştir. Sonuç olarak hasat peryodu sonuna doğru meyvelerin küçülmesine karşı içeriklerindeki fenolik bileşikler ile antioksidant kapasitenin arttığı tespit edilmiştir. Geç dönemde hasat edilen dutların erken dönemde hasat edilenlere göre insan sağlığı açısından daha faydalı olabilecekleri düşünülmektedir.

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* Corresponding author
E-mail address: mpehluvan@gmail.com (M. Pehluvan)

Introduction

Mulberry, widely distributed in Asia, Europe, North America, South America, and Africa, belongs to the genus *Morus* of the family Moraceae. For more than 5000 years, it has been planted for sericulture and has been a valuable resource. These fruit species can cultivate a wide range of ecological conditions, which can affect the chemical composition and nutritional status of plants (Ercişli and Orhan, 2006; Arabshahi-Delouee and Urooj, 2006).]. In Turkey, mulberries are widely grown in the central and the east regions, where they are consumed it as fresh, dried, turned into juices, jams and marmalades or prepared as local products such as pekmez, pestil or cevizli sucuk. Traditionally, mulberries have also been made use of medicinal purposes in Turkey. This traditional use indicates the ancestral knowledge of the great health-promoting properties of these fruit, resulting from their high contents in bioactive compounds (Ercişli and Orhan, 2006; Ercişli and Orhan, 2008) which have been shown to display some variation according to the *Morus* genotype (Gerasopoulos and Stavroulakis, 1997; Lee ve ark., 2004).

Mulberry fruits are rich in organic acids and its nature and concentration are important factors influencing the organoleptic properties and can maintain the nutritive value of fruit (Daood ve ark., 1994). Studies have been reported on the chemical composition and nutritional potentials of some mulberry species worldwide (Ercişli ve Orhan, 2006; Arabshahi-Delouee and Urooj, 2006; Ercişli and Orhan, 2008; Huang ve ark., 2013).

Mineral composition with five day intervals determination in a harvest season, have been recently studied and found to be some fluctuation during harvest season in white mulberry (*Morus alba* L.) fruit (Karlıdağ ve ark., 2012), but we are not aware of any additional published study on bioactive compounds (phenolics and antioxidant capacity) and organic acids of these fruit. Mulberry trees can be harvested many times for their fruit in a long harvest season which takes from 15 days to over 2 months. Therefore, this study was carried out for the purpose of evaluating the status of some important phenolic compounds, antioxidant capacity and organic acids throughout a harvest season in two mulberry species.

Material and Method

Plant Material

The study area is located in the east part of Turkey, Aras Valley, varying from 877 to 1497 meters above sea level (m a.s.l., Table 1). For each location, one tree per species (*Morus nigra* L. and *M. alba* L.) was selected as plant material. Because of grown from seed and ungrafted material, each tree was a different genotype within species. Therefore, locations were separately evaluated in terms of harvest dates. Different harvest dates were July 1, July 15 and August 1, 2012 as fifteen day intervals. Fruit was collected from one tree for each species at three harvest dates in each location. Samples were selected for uniform shape and colour and transported immediately to the laboratory for analyses.

Table 1. Locations of sample collection in Aras Valley.

Location name	Longitude	Latitude	Altitude
Iğdır	N39°56'40.0''	E 44°06'12.72''	877 m a.s.l.
Tuzluca	N 39°57'32.64''	E 43°39'34.38''	1497 m a.s.l.
Kağızman	N 40°08'46.38''	E 43°07'38.10''	1339 m a.s.l.

Standard Quality Parameters

Standard quality parameters were determined on 30 fruits per each species at different harvest dates. Fruit weight was determined with an electronic balance (0,01 g accuracy). Fruit width and length were measured with a digital calliper (0,01 mm accuracy). Titratable acidity (TA), pH, and soluble solids content (SSC) were assessed in juice pressed from the whole fruit (10 fruits per replicate x 3 replicates). TA was determined in 10 mL fruit juice by diluting in 10 mL distilled water and titrating with 0.1 N NaOH to pH 8.1 (AOAC, 1984), and expressed as g malic acid 100 ml^{-1}. A digital table refractometer (WAY-2S, Seoul, South Korea) was used for SSC measurement and data given as °Brix. The pH of fruit juice was determined using a portable pH meter (Jenco Instruments Inc., San Diego, USA).

Extraction and Determination of Organic Acids and Phenolic Compounds

High Performance Liquid Chromatography (HPLC) system included an LC-20 AT pump, a CTO-20A column oven, and a SPDM20A prominence diode-array detector were used as an apparatus, and equipped with a SIL-20A HT auto sampler (Shimadzu Corp., Kyoto, Japan). The LabSolutions LC (Shimadzu) software was used for collecting and processing data, obtained through reading different wavelengths.

The method of organic acid extraction and determination as in Bevilacqua and A. N. Califano (1989) was carried out by some minor modifications. About 150 g samples from each species per trial unit were fragmented and 5 g was transferred into centrifuge tubes. The samples were supplemented with a 10 ml of 0,005 N H_2SO_4 and were homogenized. After that, the samples were mixed for one hour with a shaker and centrifuged at 15,000 x g for 15 min. 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 acid separation was carried out using an Agilent Hi-Plex H (8 µm, 300 mm 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. Column oven temperature was adjusted 55 °C. The mobile phase was filtered through membrane filter (47 mm, 0,45 µm) and was sonicated for 10 min in an ultrasonic bath 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. Organic acids (Citric acid, Tartaric acid and Malic acid) were quantified from regression curves calculated for standards purchased from Sigma-Aldrich (Steinheim, Germany). The concentrations of organic acids for samples are represented as g $100g^{-1}$ fresh weight (fw).

The phenolic compounds were determined method described in Rodrigez-Delgato ve ark (2001). About 150 g of samples were fragmented and 5 g from each sample was transferred to centrifuge tubes. The samples were mixed homogenously then diluted 1:1 with distilled water and centrifuged at 15,000×g for 15 min. The supernatant was passed through 0,45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA), then injected into HPLC system (gradient). The chromatographic separation was achieved with 250 mm×4,6 mm, 5 µm AEC column (UK). The following solvents in water with a flow rate of 1ml/min and 20 µl injection volume were used for spectral measurements at 254 and 280 nm: as mobile phase solvent A, Methanol–Acetic acid–Water (10:2:88) and Solvent B, Methanol–Acetic acid–Water (90:2:8). Individual phenolic acids (Gallic, Chlorogenic, Caffeic acid, *p*-coumaric, Rutin) were quantified from regression curves calculated for authentic standards purchased from Sigma-Aldrich (Steinheim, Germany). Concentration data are presented as mg $100g^{-1}$ fresh weight (fw).

Trolox Equivalent Antioxidant Capacity

The assay of trolox equivalent antioxidant capacity (TEAC) was performed according to Re ve ark (1989). $ABTS^{*+}$ was generated by oxidation of ABTS salt 7mM with $K_2S_2O_8$ 2.45 mM and allowing the mixture in the dark at room temperature for 12–16 h. For longer stability, 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 (Özgen ve ark., 2006). After addition of 3 ml of diluted $ABTS^{*+}$ solution to 30 µl of fruit sample (extracted with ethanol: frui juice/ethanol, v/v, 1:10) or trolox standards, the absorbance was taken 6 min after initial mixing. The percentage inhibition of absorbance of $ABTS^{*+}$ at 734 nm was then calculated and plotted towards concentrations of test/standard substances as a function of time or concentration. The TEAC value was finally calculated as the ratio of the slopes of the linear regression of the concentration response curves of the test substances towards the reference substance (Trolox). The results are expressed as µmol trolox equivalent (TE) g^{-1} fresh weight (fw).

Statistical Analysis

All determinations were done in triplicate. For each species per location, means were tested for statistical differences among harvest dates by analysis of variance, using JMP 5.1 software (JMP, A Business Unit of SAS, Cary, NC, 2003), followed by the Fisher's least significant difference (LSD) test at $P \leq 0,05$.

Result and Discussion

Standard quality parameters were determined for black and white mulberries at different harvest dates in three locations as shown Table 2. Generally, white mulberries were in average heavier, less acid and sweeter as shown by titratable acidity, SSC and juice pH values. Fruit weight, width and length decreased in both black and white mulberry while titratable acidity increased in from first harvest date (July 1) to last harvest (August 1). Some fluctuations were observed SSC and fruit juice pH during the harvest season. Decreased fruit weight may be linked to high temperature and raised solar radiation at the end of harvest season. These stuation may affect plant water quantity and reduce photosynthesis efficiency. Fruit white and SSC were also found to be significantly different in white mulberry during harvest season (examined at five day intervarls) in a previous study (Karlıdağ ve ark., 2012).

Organic acids found in fruits have no negative effects on human body, as they are rapidly oxidized during the metabolisation. Some of them are Citric, Malic and Tartaric acid and are predominant in most fruit species (Schobinger, 1988; Cemeroğlu, 2004). Citric, Tartaric and Malic acid were investigated and determined in both white and black mulberry during harvest season in three locations as shown Table 3. Malic acid for white mulberry and Citric acid for black mulberry predominated quantitatively in the current study and these were in accordance with a previous report (Bozhüyük ve ark., 2015). The date of harvest, August 1, 2012 gave the highest content of Citric acid in three locations for white mulberry and in two locations for black mulberry. There was found to be fluctuations the content of Citric acid in *M. nigra* in Tuzluca location. The same results were obtained in Tartaric acid. Only some fluctuation was observed among harvest dates in terms of the content of Tartaric acid for *M. nigra* in the location of Tuzluca. Alteration in the content of organic acids during harvest period may depend on environmental factors affect fruit ripening on the accumulation of primary metabolists.

Table 2. Some physical and chemical quality attributes of black and white mulberry fruit at different harvest dates. Values represent means of three (pH, titratable acidity TA, soluble solid content SSC) or 30 replicates. Mean values followed by a different letter within the same column in each location are significantly different at P ≤ 0,05 (LSD test).

Harvest Dates	Fruit Weight (g)		Fruit Width (mm)		Fruit Length (mm)		SSC (%)		Titratable Acidity (g 100 mL^{-1})		pH	
	M. alba	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*
						Iğdır Location						
July 1	4.51a	3.79a	15.86a	16.72a	26.40a	22.35a	20.23b	16.10b	0.27c	0.59b	5.78b	4.38c
July 15	4.00b	3.76a	15.19ab	15.48a	25.91a	20.56a	20.23b	18.63a	0.32b	0.52c	5.79b	4.67a
August 1	3.98b	2.97b	14.78b	13.14b	24.70a	21.20a	21.17a	14.27c	0.36a	0.64a	5.92a	4.55b
						Tuzluca Location						
July 1	3.48a	3.26a	16.90a	15.89a	22.82a	24.92a	18.13b	15.13b	0.31b	0.78b	6.05ab	4.31b
July 15	2.52c	2.90b	14.15c	16.01a	19.19c	23.48a	17.27c	17.57a	0.23c	0.35c	6.04b	5.01a
August 1	3.00b	2.26c	15.16b	13.22b	21.10b	23.10a	21.30a	11.83c	0.36a	1.34a	6.09a	3.78c
						Kağızman Location						
July 1	3.38a	2.44a	16.32a	14.74a	24.29a	23.11a	19.80c	20.20b	0.21c	0.16b	5.69a	5.56a
July 15	2.92b	2.14b	13.51b	12.18b	20.88b	19.75b	27.83b	19.87c	0.29b	0.16b	5.55c	5.46b
August 1	2.30c	1.68c	13.57b	12.76b	19.67b	18.33c	29.90a	27.23a	0.34a	0.18a	5.65b	5.55a

Table 3. Some organic acid and TEAC of black and white mulberry fruit at different harvest dates. Values represent means of three replicates. Mean values followed by a different letter within thesame column in each location are significantly different at P ≤ 0,05 (LSD test)

Harvest Dates	Citric Acid (g 100g^{-1})		Tartaric Acid (g 100g^{-1})		Malic Acid (g 100g^{-1})		Trolox Equivalent Antioxidant Capacity (µmol g^{-1})	
	M. alba	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*
	Iğdır Location							
July 1	2.14 b	10.73 b	0.01 b	0.11 c	9.40 a	4.65 b	0.96 b.	4.25 c
July 15	1.61 c	7.01 c	0.12 b	0.14 b	7.81 b	3.66 c	1.02 ab	6.93 b
August 1	3.32 a	12.13 a	0.19 a	0.15 a	9.64 a	5.14 a	1.06 a	7.89 a
	Tuzluca Location							
July 1	0.97 c	12.25 a	0.01 c	0.18 a	4.71 a	4.12 a	0.94 b	4.92 c
July 15	1.39 b	5.63 c	0.04 a	0.16 b	4.29 b	3.53 b	1.01 ab	9.19 a
August 1	1.51 a	8.85 b	0.03 b	0.16 b	4.97 a	1.05 c	1.06 a	8.08 b
	Kağızman Location							
July 1	1.32 c	1.82 c	0.05 b	0.09 b	3.55 b	4.34 b	0.91 b	4.03 c
July 15	1.89 b	3.53 b	0.07 a	0.09 b	4.32 a	6.22 a	1.09 a	4.65 b
August 1	2.24 a	5.23 a	0.07 a	0.20 a	2.75 c	4.17 b	1.25 a	7.43 a

Trolox equivalent antioxidant capacity (TEAC) also summarized as shown Table 3. The highest TEAC value obtained from the latest harvest date, August 1 in three locations for *M. alba* and in two location (Iğdır and Tuzluca) for *M. nigra*. Second and third harvest gave more TEAC values than first harvest date in Tuzluca location for M. nigra. The highest TEAC value obtained from the date of July 15. Data show that TEAC values at different harvest dates and in three locations were in all cases higher for black mulberry than for white mulberry. Previously, similar result has been reported between black and white mulberries in a study (Gündoğdu ve ark., 2011). The fate of phenolic compounds during different harvest dates in three locations in both black and white mulberry species was investigated. The assessment of some phenolic acids is summarized Table 4. Data demonstrate that the concentration of Gallic acid, Chlorogenic acid, Caffeic acid, *p*-coumaric acid and Rutin at different harvest dates and in different locations were higher in black than in white mulberry fruits and it was in accordance with those reported by Pehluvan ve ark (2015). Generally speaking, the concentration of all investigated individual phenolics quantitatively increased at second and third harvest dates

compared to first harvest date. In these increases, some fluctuation was observed the concentration of rutin for *M. nigra* in Tuzluca location.

Phenolic compounds of most fruits are influenced by growing location and climatic conditions (soil nutrient, water, day and night temparetures, and sunlight) is well known (Zorenc ve ark., 2016). Morever, plants synthesis antioxidants that are used in their deffence system against oxidative stress (Kalt ve ark., 2001; Lata ve ark., 2005). In the present study, the alteration of investigated phenolic compounds and antioxidant capacity in mulberry species may depend on environmental stress factors. Individual phenolic compouns and antioxidant capacity increase in the fruit samples at the end of harvest season. These increase may be explained by rising of between day and night temperature difference or declining of soil humidity.

Table 4. Some individual phenolic content of black and white mulberry fruit at different harvest dates. Values represent means of three replicates. Mean values followed by a different letter within the same column in each location are significantly different at P ≤ 0,05 (LSD test).

Harvest Date	Gallic Acid (mg 100 g^{-1})		Chlorogenic Acid (mg 100 g^{-1})		Caffeic Acid (mg 100 g^{-1})		*p*-Coumaric Acid (mg 100 g^{-1})		Rutin (mg 100 g^{-1})	
	M. alba	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*	*M. alba*	*M. nigra*
	Iğdır Location									
July 1	0.31 c	0.51 c	11.75 b	7.02 c	0.37 c	1.38 c	1.15 c	1.37 c	3.42 c	1.81 c
July 15	0.36 b	0.80 b	11.71 b	13.83 b	0.81 b	3.19 b	1.98 a	2.37 b	4.50 b	4.79 b
August 1	0.61 a	1.55 a	12.04 a	25.19 a	1.69 a	6.38 a	1.45 b	4.48 a	4.78 a	8.78 a
	Tuzluca Location									
July 1	0.57^{ns}	0.58 c	11.81 c	16.52 c	0.54 c	2.67 b	1.55 a	2.02 b	2.89 b	6.89 c
July 15	0.58	1.14 b	12.59 b	19.92 a	0.62 b	4.89 a	0.78 b	4.01 a	4.68 a	15.68 a
August 1	0.64	1.22 a	13.01 a	18.99 b	0.71 a	3.08 b	0.75 b	2.18 b	4.80 a	8.78 b
	Kağızman Location									
July 1	0.58 c	0.53 b	11.68 c	13.60 c	0.41 c	4.24 b	1.20 b	4.06 c	3.12 c	9.54 c
July 15	0.68 b	0.70 b	13.23 b	17.42 b	1.07 a	5.86 a	1.32 b	4.32 b	6.76 a	16.78 b
August 1	1.12 a	1.11 a	15.73 a	20.75 a	0.97 b	6.76 a	1.53 a	5.95 a	4.63 b	24.76 a

Conclusion

In this study, the content of all investigated phenolic compounds and antioxidant capacity was higher in black than in white mulberry at all harvest dates per each location. Citric acid in black and Malic acid in white mulberry predominated. Generally speaking, fruit weight and its dimension decreased from the beginning harvest to end while with some fluctuation, SSC was increased. Health promoting compounds like individual phenolics, organic acids and antioxidants in mulberries also increased towards the harvest season. Contrary to get smaller fruit size, its phenolic compounds and antioxidant capacity increased towards the end of harvest season. As a result of the study, middle or late harvested mulberry fruits may be valuable for human health compared to earlier ones in terms of health promoting compounds.

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