

Effect of Genotypes on Chemical and Physical Properties of Mulberry

Dutların Fiziksel ve Kimyasal Özellikleri Üzerine Genetopinin Etkisi

Research Article

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ABSTRACT

In this research, the physico-chemical properties and mineral composition of white (*Morus alba* L.), black (*M. nigra* L.) and urmu (*M. nigra* L.) mulberries, grown in Southeast Anatolia region of Turkey, were investigated. Urmu contained the highest total anthocyanin and phenolic compounds (1071 mg cyanidin-3-glucoside equivalents, and 1456 mg gallic acid equivalents/100 g fresh matter respectively), and white mulberry had the highest HMF and browning index (36.15 mg kg⁻¹ and 17.61 respectively). Maximum L and b values were observed in white mulberry. The mean values of mineral compositions of the mulberry types in 100 g fresh fruit were 238 mg P, 1138 mg K, 139 mg Ca, 116 mg Mg, 6 mg Fe, 0.8 mg Cu, 4.3 mg Mn and 3.9 mg Zn. The highest total flavonoid and ascorbic acid were determined in urmu mulberry (312 mg quercetin equivalents/100 g fresh matter and 32 mg/100 g).

Key Words

Anthocyanins, HMF, minerals, phenolics.

ÖZET

Bu çalışmada, Türkiye'nin Güneydoğu Anadolu bölgesinde yetiştirilen beyaz (*Morus alba* L.), siyah (*M. nigra* L.) ve urmulu (*M. nigra* L.) dutların fiziko-kimyasal özellikleri ve mineral bileşimi araştırılmıştır. Urmulu dutlar en yüksek toplam antosiyanin ve fenolik bileşikler içerirken (1071 mg siyanidin-3-glikozit eşdeğerleri, ve 1456 mg gallik asit /100 g taze madde sırasıyla eşdeğer) ve beyaz dut en yüksek HMF ve esmerleşme indeksine (36.15 mg kg⁻¹ ve 17.61 sırasıyla) sahiptir. Maksimum L ve b değerleri beyaz dutlarda gözlenmiştir. 100 gram taze dut meyvesi çeşitlerinin mineral bileşimlerinin ortalama değerleri 238 mg P, 1138 mg K, 139 mg Ca, Mg 116 mg, 6 mg Fe, 0.8 mg Cu, 4.3 mg Mn ve 3.9 mg Zn. En yüksek toplam flavonoid ve askorbik asit (312 mg kuarsetin/100 g taze madde ve 32 mg eşdeğer/100 g) urmulu dutta belirlenmiştir.

Anahtar Kelimeler

Antosiyaninler, HMF, mineraller, fenolikler.

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INTRODUCTION

Traditionally, deep coloured fruits and vegetables are considered as healthier for human body, especially in oriental countries. The mulberry belongs to the genus *Morus* of the family Moraceae. There are 24 species of *Morus* and one subspecies, with at least 100 known varieties. Mulberry (*Morus* sp.) has deep coloured fruits. Due to its high adaptability, mulberry is grown in the diverse climatic and soil conditions, especially in chalky and clay soils, and is resistant to drought to some extent.

Around 2.5 million mulberry trees are naturally grown in different regions of Turkey, and annually 75 thousand tons of mulberry fruit is harvested between June and August. The main mulberry production area in Turkey is Black Sea region followed by eastern and central regions.

There are, economically important, three types of mulberry that grown in Turkey: 95% *Morus alba* L. (white), 3% *M. nigra* L. (black) and 2% *M. rubra* L. (red). *M. nigra* has two types of fruit, one is black the other is reddish-black known as urmu [1-5]. The mulberry fruits are consumed either as fresh or processed in diverse products. Fresh mulberries are very perishable; its transportation and marketing is very difficult, and can only be stored for maximum of six weeks in cold conditions. For longer storage, further processing is required [5]. Mulberry fruit can be processed into jams, marmalades, juices, syrups, natural dyes for cosmetic and medicine industry in Turkey [1]. Though there are many studies on various mulberry types in general, limited comparative study is available concerning the physico-chemical and mineral composition of white, black and urmu mulberry types that grown in the same ecological conditions. The objective of this research is to determine and compare some physico-chemical characteristics and mineral compositions of these mulberry types.

MATERIALS and METHODS

Materials

The samples from white, red and black mulberry types were harvested by hand in Adiyaman province, Southeast Anatolia, Turkey, between 5th

and 20th of July. The samples were kept at -20°C for further studies.

Chemicals and Reagents

Folin-Ciocalteu reagent, 2,2-diphenyl-1-picryl hydrazyl (DPPH), 5-(hydroxymethyl)-2-furaldehyde, barbituric acid, p-toluidine, potassium metabisulfite, sodium hydroxide, potassium chloride, sodium acetate, ascorbic acid, meta-phosphoric acid, oxalic acid, catechin, sodium nitrate, aluminum chloride, n-hexane, nitric acid and hydrogen peroxide were supplied from Sigma-Aldrich (St. Louis, MO, USA). Distilled water, for the HPLC mobile phase and all analytical steps were produced in an Elix 5 water purification system (Millipore, Molsheim, France).

Apparatus

Fruit colours were measured using a CR-400 chromometer (Konica Minolta, Japan). UV-VIS lambda 25 spectrophotometer was used to determine ascorbic acid, HMF, total anthocyanins, total phenolics, total flavonoids and browning index (Perkin Elmer, Waltham, MA, USA). ICP OES Optima 2100 was used to determine mineral element content (Perkin Elmer, Waltham, MA, USA).

Methods

Preparation of the Mulberries Extract

5 gr fresh fruit was homogenised in 100 ml ethanol-water (1:1, v/v) in a blender. The mixture was subjected to an ultrasonic bath for 60 min. The suspension was filtered through Whatman No. 1 filter paper (Sigma-Aldrich, St. Louis, Missouri, USA). The extracts were stored in a refrigerator till the analyses were carried out.

Physicochemical Properties

The samples were analysed for fruit weight, fruit colour, pH, total soluble solid content as outlined by [6]. The pH was measured using digital pH-meter (WTW Inolab, Weilheim, Germany). Titratable acidity was determined potentiometrically titrating the samples with 0.1 N NaOH, and expressed as percent citric acid. Total dry matter was determined gravimetrically. Total soluble solid content (TSS) was measured using Abbe refractometer (250 HE, Kyoto, Japan) at 20°C.

Measurements of the Mulberryfruit's Color

Fruit colour was measured using a CR-400 chromometer (Konica Minolta, Japan), and result were recorded as L (lightness), +a (redness) and +b (yellowness). The colorimeter was calibrated with white reference layer (Number: 14533046) before measurement [7].

Determination of the Total Anthocyanin Content

Total anthocyanin content was determined by the pH differential method. Briefly, pH 1.0 buffer was prepared by using 0.2 M KCl and 0.2 N HCl solutions. Buffer at pH 4.5 was prepared by using sodium acetate and pH was adjusted with acetic acid. Absorbance was measured at 520 and 700 nm using UV-VIS spectrophotometer.

Data were expressed as mg/100 g cyaniding-3-glucoside fresh matter. The absorbance of each sample was calculated using the following equation [6,8]:

$$A = (A_{510 \text{ pH } 1.0} - A_{700 \text{ pH } 1.0}) - (A_{510 \text{ pH } 4.5} - A_{700 \text{ pH } 4.5}) \quad [1]$$

Determination of HMF

10 grams of fruit was dissolved in 20 ml water, and transferred to a 50 volumetric flask. 2 ml of the solution and 5.0 ml of p-toluidine solution were transferred into two test tubes; to the first tube 1 ml of distilled water was added (reference solution); to the second 1 ml of barbituric acid solution 0.5% (sample solution). The absorbance of the solutions at 285 nm was determined using a Perkin Elmer UV-visible spectrophotometer [9].

Determination of Total Flavonoid

Total flavonoid content was determined using a spectrophotometric method based on formation of flavonoid complex with aluminum. 3 mL deionized water and 0.3 mL NaNO₂ were added to both extract (1 mL) and standard catechin solution (50-500 mg L⁻¹). After keeping 5 min at room temperature, 3 mL of 2% AlCl₃ solution was added; waited for 5 min and then 2 mL of 1 M NaOH was added. The solution was then made up with deionized water to 10 mL. The absorbance was measured at 510 nm. Total flavonoid content was calculated as milligrams of catechin equivalent (CE) per kilogram of fresh fruit using standart curve [10].

Determination of Browning Index

To obtain clarify the samples, ethyl alcohol was added, and then filtered and centrifuged. Potasium metabisulfide was added to the resulting samples and absorbance was determined at 420 nm [6].

Determination of Total Phenolic

Total phenolic compounds were measured using Folin-Ciocalteu and expressed as mg gallic acid per liter [11].

Determination of Total Fat Content

Approximately 5 g (a) fine grinded and homogenized samples were weighed on filter paper (1 mm) and put in soxhalet capsules. Clean and dry fat beakers were weighed (b). Soxhalet capsules were placed into soxhalet instrument. 2/3 of soxhalet beakers were filled with hexane or petrolium ether. Soxhalet beakers were placed into the soxhalet. Suitable program for the choosen solvent was selected for extraction. After extraction, soxhalet beakers were placed into oven at 105°C for 1 hour. The dried soxhalet beakers were cooled and weighed (c) (TFC, 2004). The fat contents were calculated using equation below:

$$\% \text{ Total fat content} = [(c - b)/a] \times 100 \quad [2]$$

Determination of Ascorbic Acid

200-300 g sample (W) was weighed into a blender, and the same amount extraction solution (acetic acid solution 8%) was added to sample. Samples and solutions were homogenized using blender for 2 minutes. 10-40 g of homogenized mixture was transferred to the 100 ml rounded flask and filled upto 100 ml. with extraction solution. Flask was shaken vigorously, and then the solution was filtered. 5-20 mL of filtrate was placed into 50 mL erlenmayer flask. And the solution was titrated with 2,6 dichlorophenolindophenol (V) [12]. Amount of ascorbic acid was calculated as below;

$$\text{Ascorbic acid (mg/100 g)} = V.F.100/W \quad [3]$$

Determination of Mineral Content

Analysis for P, K, Ca, Mg, Na, Fe, Cu, Mn and Zn were determined using ICP-OES Perkin Elmer Optima 2100. The samples were homogenized using blenders: 1 g of sample was transferred into

the microwave oven vessels, on which 6 mL HNO₃ and 1 mL of H₂O₂ were added. After keeping for 10-15 minutes for pre-ashing, the vessels was placed in microwave oven, and digested upto 200°C under pressure for 1 hour. The digested samples were taken into sample tubes and diluted to 15 mL with ultra pure water. The sample tubes were placed into auto sampler of ICP-OES. Amount of each mineral was calculated using calibration curve of corresponding mineral. Conditions for the ICP-OES were: plasma 15 L/min, auxiliary 0.2 17 min, nebulizer flow: 0.55 17 min, power of RF generator 1500 watt and sample flow rate 2 mL/min [13].

Statistical Analysis

The trial was arranged according to factorial design in completely randomised blocks. All the analyses were carried out triplicate. The data obtained were subjected to analysis of variance, the significant differences were tested using Duncan multiple ranges test ($p < 0.05$) to determine the differences among treatments [14].

RESULTS and DISCUSSION

Fruit Weight, Width, Length, Stalk, pH, Total Soluble Solid Content, Total Dry Weight

The fruit weight, width and length, stalk and fruit colour, pH, acidity, total soluble solid content (TSS) and total dry weight (TDW) of mulberry samples were given in Table 1. A total of 50 mulberry fruits were used to measure mean weight, stalk, width and length. The weight, width, length and stalk of mulberry types ranged between 3.85-4.08 gram, 16.82-17.87 mm, 25.62-29.82 mm and 3.76-4.01 mm respectively, with urmu mulberry having the biggest fruits. The pH ranged from 3.52 (urmu) to 5.40 (white), acidity from 0.14 (white), to 1.37 (urmu), TSS from 21.13 (white), to 25.13 (urmu), TDW from 62.22 (white) to 75.95 (urmu). The L value ranged from +14.04 (black) to +77.04 (white), a value from +6.54 (black) to 11.04 (urmu), and b value from 1.98 (black) to 15.01 (white).

According to the results, because of its higher TSS and TDW content, bigger and attractive colorful appearance, we can recommend urmu type mulberry for both processing fresh marketing. In a similar research, [1] determined

fruit weight of mulberries between 2.14 g and 4.37 g, with *M. nigra* having the biggest fruits. The moisture contents were from 71.5% (*M. alba*) to 74.6% (*M. rubra*), pH from 3.52 (*M. nigra*) to 5.60 (*M. alba*), acidity from 0.25% (*M. alba*) to 1.40% (*M. nigra*), TSS from 15.9% (*M. rubra*) to 20.4% (*M. alba*), and TDW from 24.41% (*M. rubra*) to 29.50% (*M. alba*). Fruit colour was determined as L value, from +14.3 (*M. nigra*) to +78.4 (*M. alba*), a value from -13.6 (*M. alba*) to 8.55 (*M. rubra*), and b value from 1.72 (*M. nigra*) to 16.2 (*M. alba*).

Total Phenolic, Total Anthocyanin, Total Flavonoid and Ascorbic Acid Content of Mulberries

Total phenolic, total anthocyanin, total flavonoid and ascorbic acid content of mulberry types were given in Table 2. The content of total phenolic compounds and flavonoids depends on geographic location and soil on which the mulberry trees are grown, and plant genotype and cultivation conditions [1,15]. The content of total phenolic compounds were determined in urmu, black and white mulberries as 1456, 1006 and 401 mg kg⁻¹ (expressed as GAE equivalent), respectively. [1], determined a similar content of total phenolics in the fresh black (1422 mg.kg⁻¹), red (1035 mg.kg⁻¹) and white (181 mg.kg⁻¹) mulberries. In the other studies; total phenolics in the fresh white and black mulberries were found 1175.3 and 1451.4 mg.kg⁻¹, respectively [5,15]. Total flavonoids (expressed as CE equivalent) in urmu, black and white mulberries were 312, 290 and 41 mg.kg⁻¹, respectively. [15] determined 599.7 mg kg⁻¹ total flavanoids in the fresh white mulberries. [1] determined a similar content of total flavanoids in the fresh black (276 mg.kg⁻¹), red (219 mg.kg⁻¹) and white (29 mg.kg⁻¹) mulberries. [5] found total flavanoid content of black mulberries 768.7 mg kg⁻¹.

We determined the total monomeric anthocyanin content of urmu, black and white mulberries as 1072, 839 and 91 mg/100 g dry weight. [5] found anthocyanin in black mulberries 1221 mg/100 g dry weight (expressed as cyaniding -3-glucoside). The difference between the results may be attributed to fruit type and maturation period. Anthocyanin content of fruit is reported to change during ripening period. Over ripened,

Table 1. Some characteristics of Mulberries.

Species	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Fruit stalk (mm)	Fruit color			pH	Acidity (%)	Total Soluble Solids (%)	Total Dry Weight (%)
					L	a	b				
Urmu Mulberry	4.08a	17.87a	29.82a	4.01a	27.04b	11.04a	2.03b	3.52c	1.37a	25.13a	77.95a
White Mulberry	3.85c	16.82c	25.62c	3.76c	77.04a	10.70c	15.01a	5.40a	0.14c	21.13c	62.22c
Black Mulberry	3.96b	16.98b	27.45b	3.94b	14.04c	6.54b	1.98b	4.51b	1.17b	22.92b	65.95b

Values in the same column with different lower-case letters are significantly different at $P < 0.05$.

Table 2. Total phenolics, anthocyanins, flavonoid and ascorbic acid content of mulberries.

Species	Total phenolics (mg/kg GAE/fresh mass)	Total Anthocyanins (mg/100g dry weight)	Total Anthocyanins (mg/100 g dry weight)	Total Anthocyanins (mg/100 g dry weight)
Urmu Mulberry	1456a	1072a	312a	31.99a
White Mulberry	401c	91c	41c	19.86c
Black Mulberry	1006b	839b	290b	25.92b

Values in the same column with different lower-case letters are significantly different at $P < 0.05$.

Table 3. HMF, Browning index and total fat content of mulberries.

Species	HMF (mg/kg)	Browning Index	Total fat content (%)
Urmu Mulberry	6.77c	6.63b	0.74c
White Mulberry	36.15a	17.61a	0.99a
Black Mulberry	13.29b	4.53c	0.87b

Values in the same column with different lower-case letters are significantly different at $P < 0.05$.

Table 4. Mineral contents of mulberries.

Species	Mineral elements (mg/100 g)								
	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Urmu Mulberry	269a	1205a	156a	131a	71a	7.7a	1.2a	6.5a	4.8a
White Mulberry	211c	1055b	121c	104c	58c	4.0c	0.4c	2.5c	2.7c
Black Mulberry	236b	1155a	140b	115b	65b	5.5b	0.8b	4.0b	4.1b

Values in the same column with different letters are significantly different at $P < 0.05$.

purple-colored mulberry fruits contain the highest amount of anthocyanin, followed by purple-red and red-colored fruits. Purple colored mulberry fruits contain anthocyanin more than five times as compared to the red-colored ones [2].

Ascorbic acid contents of urmu, black and white mulberries were found as 31.99, 25.92 and 19.86 mg/100 g, respectively. [2] found increased ascorbic acid in deep colored mulberry fruits, 1.4 mg/100 g in purple, 2 mg/100 g in purple-red and 2.4 mg/100 g in red fruits.

HMF, Browning Index and Total Fat Content of Mulberries

HMF, Browning index and total fat content of mulberry types are given in Table 3. HMF contents of urmu, black and white mulberries were found as 6.77, 13.29 and 36.15 mg/kg, respectively. [16] found HMF contents of fresh black mulberry juice between 45.40-46 mg/kg. They also explained that HMF content of both black mulberry juice and concentrate increased significantly ($p < 0.01$) with higher storage temperature and time.

Browning index of urmu, black and white mulberries were 6.63, 4.53 and 17.61, respectively. Higher antioxidant content of darker fruits may

have prevented these fruits against browning reactions.

Fruits are known to contain very low amount of fat except oil fruits like olive, walnut and avocado etc. The total fat contents of the mulberries were found as 0.74% in urmu, 0.87% in black and 0.99% in white. [1] found similar values as 0.85% in *M. rubra* and 1.10% in *M. alba*. They also stated that there was a negative correlation between moisture and fat content of mulberry.

Mineral Contents of Mulberries

The mean amount of minerals in 100 g mulberry fruits were 238 mg P, 1138 mg K, 139 mg Ca, 116 mg Mg, 65 mg Na, 6 mg Fe, 0.8 mg Cu, 4.3 mg Mn and 3.9 mg Zn (Table 4). [1] reported similar result in various mulberry types.

As seen in Table 4, urmu mulberries had highest values for all the minerals investigated, but white mulberries had lowest values. K was the predominant, and Zn was the least mineral in the present study. The mineral composition of fruits depends on the type or variety and the growing conditions, such as soil and geographical conditions.

CONCLUSIONS

In the light of present study, increased levels of phenolic compounds, flavonoids, anthocyanin and ascorbic acid were found in deep - colored black and reddish black types. They also showed lower level of HMF and browning index propably due to higher levels of these antioxidant compounds. Of the mulberry types investigated, urmu (*M. nigra* L.) which has larger fruit width, length and weight, and attractive appearance, and rich in nutritional value, it is more convenient to consume as fresh and may be processed into diverse products including jam, and be used as ingredient in food processing such as confectionary. The possibility of use of urmu as food coloring should be investigated in further studies.

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