



Araştırma Makalesi (Research Article)

Phytochemical Properties of Some Mulberry Genotypes Grown in Malatya Province

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Abstract: Mulberry fruits contain large amounts of anthocyanins, polyphenols, flavonoids components and minerals which are important for human health. In this study, phytochemical properties of some mulberry genotypes in theecologicalconditions of the Malatya region were investigated. ‘Kenmochi’, ‘Bursa Siyah’, ‘44 BA 05’, ‘Elazığ İçme’, ‘23 MRK 09’ the mulberry genotypes and one patented ‘Ichinose’ cultivar were studied concerning their total phenolic content, total monomeric anthocyanin, total antioxidant capacities and individual sugar compositions. Some mulberry genotypes were found the richest with regard to total phenolic and total anthocyanins content. The total phenolic content was found from 958.60 to 3573.79 mg/kg gallic acid equivalents, total anthocyanin content to vary from 177.10 to 2221.60 mg/kg cyanidin-3-glucoside. The antioxidant activities of ‘Kenmochi’ and ‘44 BA 05’ genotypes were higher than those of the others genotypes. These results will be useful for selecting mulberry species that are important in terms of phytochemical content.

Malatya İlinde Yetişen Bazı Dut Genotiplerinin Fitokimyasal Özellikleri

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Anahtar kelimeler

Antosiyanin,

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Öz: Dut meyvesi, insan sağlığı için önemli antosiyanin, polifenol, flavonoid bileşenlerini ve mineral maddelerini yüksek miktarda içermektedir. Bu çalışmada, Malatya bölgesinin ekolojik koşullarındaki bazı dut genotiplerinin fitokimyasal özellikleri incelenmiştir. ‘Kenmochi’, ‘Bursa Siyah’, ‘44 BA 05’ ‘Elazığ İçme’, ‘23 MRK 09’ dut genotiplerivle tescillenmiş bir çeşit olan ‘Ichinose’ toplam fenolik madde içeriği, toplam monomeric antosiyanin, toplam antioksidan kapasite ve şeker bileşimleri bakımından incelenmiştir. Bazı dut genotiplerinin toplam fenolik ve toplam antosiyanin içerikleri bakımından daha zengin olduğu belirlenmiştir. Toplam fenolik madde içeriği 958.60 ile 3573.79 (mg/kg gallik asit eşdeğeri), toplam antosiyanin içeriği ise 177.10 ile 2221.60 (mg/kg siyanidin-3-glukozit) aralığında değişmektedir. ‘Kenmochi’ ve ‘44 BA 05’ genotiplerinin, antioksidan aktiviteleri bakımından diğer genotiplerden daha yüksek aktivite gösterdiği belirlenmiştir. Bu sonuçların, fitokimyasal içerik bakımından önemli olan dut genotiplerinin seçilmesinde faydalı olacağı düşünülmektedir.

1. Introduction

Fruits contain the nutrients necessary for the improvement of the human body, protection from diseases and the good functioning of many organs. The mulberry, which exists is in small fruits, has an important place in nutrition due to the high nutrient content, and interest in the mulberry is increasing recently. Mulberry belongs to the *Moraceae* family. The most commonly occurring species are *Morus*

nigra (Black mulberry), *Morus rubra* (Red-purple mulberry) and *Morus alba* (White mulberry) but the color of mulberry fruits is not enough to describe mulberry species (Aramwit et al., 2010; Ercişli and Orhan, 2007; Khalifa et al., 2018). *Morus rubra* is mostly grown in Mediterranean and Middle Eastern countries and these fruits particularly desirable in Turkey (Ercişli et al., 2010). The cultivation of this species has been known for many years in Turkey (Ercişli, 2004). Red-purple mulberries have attracted attention as a natural source of minerals (Akbulut and Özcan, 2009; Ercişli et al., 2010; Jiang and Nie, 2015), vitamins (Ercişli et al., 2010; Gündoğdu et al., 2018), carotenoids (Isabelle et al., 2008) and phenolic compounds (Sánchez-Salcedo et al., 2015).

Small fruits are important fruit types that are liked and consumed in various forms in the world and Turkey. Mulberry fruit is consumed by processing it in different products as well as fresh consumption. There is an increase in fresh fruit consumption due to its potential health benefits and high polyphenols content (Brand et al., 2017). People's fresh fruit consumption requests made it possible for this fruit to be placed on the market shelves in small packages.

In the last years, there is increasing research in mulberry fruits, their chemical composition and health benefits. There have been some *in vivo* and *in vitro* studies on the main nutrient components, chemical compositions, biochemical, and antioxidant properties of mulberry species grown in different regions of the Turkey.

Fruits, leaves, roots, and bark of several mulberry species have been used in traditional medicine in Turkey. Especially black fruits are used against various diseases diabetes, hypertension, anemia and mouth lesions (Özgen et al., 2009). Therefore black mulberry is more popular than other mulberry varieties. Mulberry juices are used to increase hair growth, aid in weight loss and control excessive thirst (Nazim et al., 2017).

Mulberry fruit is rich in compounds with phytochemical properties but fruit cultivars/genotypes, seasonal differences, fruits maturity, processing procedure, and geographic origin affect in the nutritional characteristics. The various genotypes of the same fruit may show some differences in terms of polyphenol and chemical compounds as in other studies (Bae and Suh, 2007; Ercişli and Orhan, 2008; Jakobek et al., 2012). Moreover, maturity stages as full-ripe and semi-mature affect the chemical properties of fruits such as ascorbic acid, total phenolic content (Mahmood et al., 2017; Nayab et al., 2020).

In general, the major phenolic groups were hydroxy benzoic and hydroxy cinnamic acid derivatives in mulberry fruits (Khalifa et al., 2018). The results of Gündoğdu et al. (2011) indicated that the highest content of phenolic acids, which mainly chlorogenic acid. Natic et al. (2015) reported the content of rutin in black-colored mulberry samples are high while the contents in the white- and pink-colored sample were low.

Our purpose in this study is to determine the phytochemical properties of some mulberry genotypes in mulberry genetic resources. Furthermore, it is to reveal that mulberry fruit is a natural source of polyphenol compounds.

2. Materials and Methods

2.1. Plant materials

Five genotypes of mulberry; including 'Kenmochi', 'Bursa Siyah', 'Elazığ İçme', '23 MRK 09' and '44 BA 05' and one cultivar 'Ichinose' were used to in this study. The fruits were collected from mulberry genetic resources parcel in Apricot Research Institute, Malatya. Mulberry fruits used in this study are red and black colored.

2.2. Fruit harvest and physicochemical features of mulberry fruits

Fruit characteristics of mulberries were measured on fresh mulberry immediately after the harvest.

The total soluble solids content was indicated with a digital refractometer (Pocket refractometer PAL-1, Atago, Tokyo) and the values were stated in °Brix.

The titratable acidity was determined by titration of an aqueous mulberry homogenate solution with 0.1 N NaOH to ultimate value pH 8.1 and the values were expressed as malic acid.

pH, the fruits were homogenized and the pH was measured in fruit juice using a digital pH meter (pH Benchtop Meter, Thermo Scientific Orion 2 Star).

Fruit weight, the fruit weight was measured by using an electronic balance (BL-320H, Shimadzu). The weight of mulberries with respect to three replications, with ten fruits in each replication.

2.3. Preparation of mulberry extracts

Mulberry fruits were harvested in full ripeness stage in Malatya, in June of 2019. Fruits were homogenized with an electric blender. A 1.0 g of homogenized samples were transferred into a test tube and were extracted by a mixture solution of the composition methanol: water: hydrochloric acid (70:29.9:0.1, v/v/v). After that, the mixture was centrifuged (10 min, 6000×g) and the obtained supernatant was taken into another tube. The extraction procedure was repeated two times and extracts were combined. The crude extract was filtered before used for analysis. Extracts were prepared daily and were kept at +4 °C until used.

2.4. Determination of total phenolic content

The total phenolic content was determined by the modified Folin Ciocalteu method (Singleton and Rossi, 1965). The results were expressed as milligrams of gallic acid equivalents (GAE) per kg of fresh weight.

2.5. Determination of antioxidant activity

The total antioxidant activity was determined by 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays.

2.5.1. DPPH antioxidant assay

The DPPH method was performed according to Thaiponga et al. (2006) with some modifications. The results were calculated as milligrams Trolox equivalents (TE)/kg of fresh weight.

2.5.2. Trolox equivalent antioxidant capacity (TEAC)

The TEAC assay is based on the inhibition of radical cation ABTS⁺, which prepared with potassium persulfate, by antioxidant compounds in samples (Singh and Singh, 2008). The absorbance of standard and samples was taken at 734 nm using the UV-spectrophotometer (UV-VIS 1208 Spectrophotometer, Shimadzu, Japan). Total antioxidant activities of the samples were expressed as milligrams Trolox equivalents (TE)/kg fresh weight (Thaipong et al., 2006; Re et al., 1999).

2.6. Total monomeric anthocyanins

Total anthocyanins were determined by pH-differential method (Lee et al., 2005) using a UV-VIS spectrophotometer (UV-VIS 1208 spectrophotometer, Shimadzu, Japan).

2.7. Content of sugar

Mulberry fruits were analyzed by using HPLC to determine the sugar content. The mulberry samples (1.0 g) were taken in a tube. 10 mL of distilled water was added and vortexed. After centrifugation, an amount was taken from the upper phase and filtered through a 0.45 µm filter (25 mm HPLC syringe Filter, PVDF, Lab marker). Analysis of samples was performed by HPLC (Shimadzu, Japan) with a reagent index detector (RI) (Llano et al., 2017; Talcott et al., 1999). The elution solvents consist of 100% ultrapure water. The separation column was used a Carbosep CHO 87C carbohydrate column (7.8x300 mm, Concise separations, San Jose, California) at 80 °C.

2.8. Statistical analysis

Statistical analysis was performed by one-way analysis of variance using the SPSS package (version 25). The differences among the genotypes were analyzed using the Duncan test at the 0.05 significance level. The correlations between the total phenolic content and antioxidant activities were tested by Pearson's coefficient.

3. Results

3.1. Some physicochemical features such as total soluble solids, pH, titratable acidity, fruit weight in mulberry fruits

Some physical and chemical characteristics of mulberry genotypes (the fruit weight, pH, total soluble solids, titratable acidity) are given in Table 1. Statistical differences ($P < 0.05$) were found in physicochemical properties among the mulberry genotypes. Fruit weight of mulberry genotypes ranged between 1.30 g and 3.03 g, with 'Kenmochi' mulberry fruits having the biggest fruit weight. The acidity from 0.19% (Bursa Siyah) to 0.45% (Elazığ İçme), pH from 6.18 (Bursa Siyah) to 4.84 (Kenmochi), TSS from 13.30% (Kenmochi) to 27.45% (Bursa Siyah).

Table 1. Fruit weight, TSS, pH and titratable acidity in mulberry fruits

Genotype/Cultivar	Fruit weight (g)	Total soluble solids (TSS,%)	pH	Titratable acidity (%)
Bursa Siyah	1.82cd	27.45a	6.18a	0.19d
Kenmochi	3.03ab	13.30e	4.84b	0.28bc
44 BA 05	2.31ab	23.90b	5.06b	0.40a
Elazığ İçme	2.61a	20.02c	5.01b	0.45a
23 MRK 09	1.30d	21.40c	5.93a	0.23cd
Ichinose	1.69bc	17.75d	4.90b	0.35ab

*Difference between means showed with the different letters within the same column are significant at 0.05 level.

3.2. Content of sugar in mulberry fruits

Individual sugar contents were identified by HPLC (Shimadzu, Japan). The results of the sugar analysis are given in Table 2.

Table 2. Content of sugar in mulberry fruits (g/100 g)

Genotype/Cultivar	Bursa Siyah	Kenmochi	44 BA 05	Elazığ İçme	23 MRK 09	Ichinose
Glucose	8.19a	5.18cd	5.72c	6.55b	6.40b	4.70d
Fructose	10.53a	6.41c	8.58b	8.49b	8.09b	5.80c

*Difference between means showed with the different letters within the same column are significant at 0.05 level.

3.3. Total phenolic content and antioxidant activity in mulberry fruits

The antioxidant activity was determined using DPPH and ABTS methods and the results show that the total antioxidant activity in mulberries ranged from 871.32-2368.76 mg TE /kg of fw for DPPH methods. The results between 743.00-4072.34 mg TE/kg of fw were obtained with the ABTS method. The phenolic contents and antioxidant activities determined are shown in Table 3. The results show that statistically significant differences were observed in mulberry genotypes.

Table 3. Total phenolics and antioxidant activity in mulberry fruits

Genotype/Cultivar	Total phenolic content (mg GAE/kg of fw)	DPPH (2,2-diphenyl-1-picrylhydrazyl) (mg TE/kg of fw)	ABTS (Trolox equivalent antioxidant capacity) (mg TE/kg of fw)
Bursa Siyah	1429.71c	1166.97d	1220.31d
Kenmochi	3568.17a	2221.60b	4072.34a
44 BA 05	3573.79a	2368.76a	3818.91b
Elazığ İçme	2203.60b	1737.07c	2442.94c
23 MRK 09	958.60d	871.32e	743.00e
Ichinose	3475.77a	2217.49b	4027.72a

*Difference between means showed with the different letters within the same column are significant at 0.05 level.

3.4. Total monomeric anthocyanin in mulberry fruits

The total anthocyanin contents were from 177.10 to 2221.60 mg/kg fresh weight of fresh fruit. The results concerning the total anthocyanins of genotypes are shown in Table 4. Genotypes 'Kenmochi' and '44 BA 05' have the highest anthocyanin content and no istatistical difference was found between these genotypes.

Table 4. Total monomeric anthocyanins in mulberry fruits

Genotype/Cultivar	Bursa Siyah	Kenmochi	44 BA 05	Elazığ İçme	23 MRK 09	Ichinose
Total monomeric anthocyanins (mg/kg fw)	177.10e	2200.24a	2221.60a	1272.15c	445.82d	2055.00b

*Difference between means showed with the different letters within the same column are significant at 0.05 level.

4. Discussion and Conclusion

The analysis of mulberry samples indicated different physicochemical properties among the genotypes/cultivar. Organic acids, sugars, total soluble solids (TSS) and their ratios, play important roles in the taste and flavor properties of fruit (Liang et al., 2012). Organic acid and sugar contents of fruits vary according to cultivars. In addition, sugar-acid composition and amounts are the primary determinants of taste attributes of fruits (Gündoğdu et al., 2014). Therefore, the acidity is an important parameter to assess the taste of mulberry fruits. Malic acid is the most predominant organic acids in mulberry fruits species (Gündoğdu et al., 2011). The 'Bursa Siyah' has the highest total soluble solids and pH value. Therefore, these properties give the 'Bursa Siyah' mulberry fruits a better taste. These results may be due to genotype difference. The findings of mulberries characteristics are showed similarities comparison with Çöçen's work (2017).

According to the results of this study, the main sugar identified were fructose (5.80-10.53 g/100 g FW) and glucose (4.70-8.19 g/100 g FW). Fructose was the dominant sugar followed by glucose, while sucrose was not detected. These results are showed similarity with those previously reported (Özgen et al., 2009; Sanchez et al., 2014). Glucose and fructose were considerably higher in fruits of genotype 'Bursa Siyah' than in fruits of other genotypes. Besides, 'Bursa Siyah' had the highest content of soluble solids content. Sugars constitute a large part of water-soluble dry substances. The sugar content in mulberries depends on many reasons such as the growing conditions and fruits maturity.

Anthocyanins, the main color pigments of mulberry fruit and important for potential health effects. The black-colored mulberries are a source of anthocyanins, which mainly consist of cyanidin 3-O-rutinoside, cyanidin 3-O-glucoside, pelargonidin 3-O-rutinoside and pelargonidin 3-O-glucoside (Khalifa et al., 2018). Cyanidin 3-O-glucoside is the highest content in mulberry fruit. Similar results have been indicated by Kim and Lee, (2020). The amount of anthocyanin increases with maturation in mulberries, but factors such as harvest time and type and fruit processing procedure are affect the amount of anthocyanins. Anthocyanins in some mulberry genotypes are very sensitive to enzymatic

oxidation, and waiting for a certain period leads to browning (Karaçalı, 2002). This characteristic feature was mostly observed in 'Bursa Siyah' genotype.

Fruits have different polyphenols composition and their antioxidant activities that may be caused differ greatly. Furthermore, various genotypes of the same fruit can indicate important differences in the phytochemical properties (Jakobek et al., 2012). Berries containing bioactive compounds reduce the oxidative reaction which can lead to various diseases. Literature indicates that antioxidant activity differs in many berries (blackberry, strawberry, raspberry, chokeberry, bilberry, and mulberry) with genotypes and harvest maturity (Mikulic-Petkovsek et al., 2012; Nayab et al., 2020). Mulberry fruits have higher polyphenols content, like other berries. '44 BA 05' had the highest content of total phenolics in mulberry fruits from different genotypes followed by 'Kenmochi' genotypes. A statistically significant difference was found between genotypes 'Ichinose', 'Kenmochi' and '44 BA 05' (the highest phenolic compound content) and '23 MRK 09' (the lowest amount).

DPPH and ABTS methods are simple and rapid. ABTS radical scavenging assay is a short procedure time (Durmaz, 2012) and ABTS⁺ radicals react rapidly with antioxidant compounds in fruits. However, if absorbance values are read before the end of there action, the results can be lower than expected. Therefore, the endpoint of there action was determined, when reduce of absorbance reached the finish (Jakobek et al., 2012). 'Kenmochi' have the highest antioxidant activities in genotypes.

In conclude, there were determined statistically significant differences between mulberry species in contents of total phenolic, total anthocyanin, total antioxidants and sugar. In addition, between total phenolic content and total antioxidant activities of mulberry genotypes were determined a high positive correlation.

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