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SOME PHYSICO-CHEMICAL CHARACTERISTICS OF BLACK MULBERRY (*MORUS NIGRA* L.) IN BITLIS

Volkan OKATAN[1], Mehmet POLAT[2], Mehmet Atilla AŞKIN[2]

[1]Uşak University, Sivaslı Vocational High School, 64800 Sivaslı, Uşak/Turkey
[2] Süleyman Demirel University, Faculty of Agriculture, Department of Horticulture, 32100 Isparta/Turkey

Corresponding author email: okatan.volkan@gmail.com

Abstract

In this study, physico-chemical properties (total soluble solid contents, pH, titratable acidity, vitamin C, antioxidant activity, total phenolic and total anthocyanins) of black mulberry (Morus nigra L.) fruits grown in the Bitlis province of Turkey were investigated. The total soluble solids content of black mulberry varies between 15.65 % (13-BIT-2) and 22.1 % (13-BIT-6), titratable acidity between 1.45 % (13-BIT-1) and 1.85 % (13-BIT-4), pH between 3.65 (13-BIT-2) and 4.12 (13-BIT-5), respectively. Ascorbic acid (vitamin C) was in the range from 18.40 (13-BIT-3) to 23.67 (13-BIT-6) mg/100 g fresh weight (FW). The highest total phenolic contents were found 1920 (13-BIT-2) to 2575 (13-BIT-7) mg of gallic acid equivalent (GAE) 100 g^{-1} fresh weight. Antioxidant capacity (DPPH) was in the range from 18.24 (13-BIT-1) to 23.18 (13-BIT-6) % and total anthocyanin content between 643 (13-BIT-4) and 826 (13-BIT-8) mg/100 g.

Key words: *antioxidant, phenolic, Morus nigra, mulberry, selection.*

INTRODUCTİON

Morus nigra L., called black mulberry is a species of flowering plant in the family *Moraceae,* native to in a wide area of tropical, subtropical, and temperate zones in Asia, Europe, North America, South America, and Africa.

The trees historically have been used for sericulture especially in east, central, and south Asia. There are at least 24 species with more than 100 known cultivars. Farmers cultivate mulberry for silkworms in the China and India but european farmers cultivate for fruit. (Pawlowska et al., 2008).

Anatolia is one of the important diversity centers of mulberries with a long cultivation history dating back to 400- 500 years ago. The most popular mulberry species with edible fruits grown in Turkey are black, white and red mulberry (Ercisli and Orhan, 2007).

Traditionally, the fruits have been processing into several products like mulberry juice, molasses, jam, vinegar and some very special products such as 'mulberry pestil', 'mulberry kome' in Turkey. All of these have significant marketing value due to its nutritive and distinct characteristics (Erturk and Gecer, 2012).

Black mulberry fruits have also been effectively used in folk medicine in Turkey for a long time to treat fever, protect liver, strengthen the joints, facilitate discharge of urine and lower blood pressure (Baytop, 1984).

Dark-coloured fruits, particularly berries (currant, honeyberry, aronia, blackberry, blueberry, mulberry, etc.) are recognized as contributors to the human health. In addition, there is increasing interest in pigment components of this group of fruits that may improve human health or lower the risk of disease (Lin and Tang, 2007). Black mulberry contains bioflavonoids that are important natural antioxidants and also contains non anthocyanin phenolics that are known to have many bioactive functions, including neuroprotective effects, which may be responsible for their medicinal properties (Ercisli and Orhan, 2007).

The aim of this research was to determine the some physico-chemical characteristics of local *Morus nigra* L. cultivars grown in Bitlis region in Eastern part of Turkey. The obtained results can be used in the registration process of these local cultivars and may be taken into consideration in the selection of parents in future breeding programs.

MATERIALS AND METHODS

Fruit material

Eight local black mulberry cultivars were harvested in different region of Bitlis, East Anatolia, Turkey, in August 2014. The harvested fruits were then transported to the laboratory for analysis.

Methods

Total soluble solid content (TSS) was measured with a digital refractometer (Model HI-96801 Hanna, German) at 20 ºC. pH measurements were done by using Hanna-HI 98103. pH meter calibrated with pH 4.0 and 7.0 buffers. Titratable acidity was determined potentiometrically by titrating the sample with 0.1 NaOH until the pH reached 8.01 and expressed as citric acid.

Determination of total phenolics

Total phenolic content were determined with Folin-Ciocalteu assay (Singleton and Rossi, 1965). For this, flesh and peels (10 g) were centrifuged at 6000 rpm after homogenized in 40 ml ethanol solution. After, diluted (1/10) 1000 µl Folin-Ciocalteu and 800 µl Na_2CO_3 solution was added upon supernatant. After 2 hours of incubation, the samples were read spectrophotometrically at the wavelength 750 nm. Water-ethanol mixture was used as blank. Gallic acid was used as standard in the calculation.

Determination of total anthocyanins

For total anthocyanin analysis, 10 g flesh and peels were homogenized in methanol solution that included HCl 1 %. After a night of standing, the samples were filtered using a filter paper. The samples were read against the blank at the wavelengths of 530 and 700 nm (Giusti and Wrolstad, 2001).

Determination of vitamin C

After pureeing and filtering, the fruit juices samples were obtained. The juices were used for vitamin C analysis. The samples were homogenized by centrifuge and 400 µL oxalic acid (0.4 %) and 4.5 ml 2,6 - diclorofenolindofenol solution were added upon supernatant. The data were read spectrophotometrically at the wavelength of 520 nm against the blank.

Determination of radical – scavenging activity

In the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) assay, antioxidants were capable to reduce the stable radical DPPH• to the yellow coloured diphenylpicrylhydrazine (DPPH-H). The test is based on the reduction of an alcoholic solution of DPPH• in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H (Gulcin, 2007). The DPPH• radical-scavenging activity was estimated after Blois (1958). Briefly, 0.1 mL of each sample extract was mixed with 0.9 mL of 0.04 mg/mL methanolic solution of DPPH•. The mixtures were left for 20 min at room temperature and its absorbance then measured at 517 nm against a blank. All measurements were carried out in triplicate. The percentage of DPPH• scavenging activity was calculated using the following equation:

$$\% \text{ DPPH} = [(A_c - A_s)/A_c] \times 100$$

where A_c was the absorbance of the negative control (contained extraction solvent instead of the sample), and A_s was the absorbance of the samples.

Statistical analysis

Five replicates including 20 fruits per replicate were used. Descriptive statistics of total soluble solid contents, pH, titratable acidity, vitamin C, antioxidant activitiy, total phenolic and total anthocyanins extracted from eight *Morus nigra* cultivars were expressed as mean ± standard error (SE). The phytochemical characteristics were statistically analyzed with One-way ANOVA with five replicates. Duncan Test determined significant differences between the evaluated cultivars. All statistical evaluations were performed using SPSS 20 program.

RESULTS AND DISCUSSIONS

Table 1 shows the results of pH, TSS (%) and titratable acidity composition. In Table 2 are presented the results regarding the analysis of vitamin C, free radical scavenging activity, total phenolic and antocyanin content of eight mulberry cultivars collected from Hizan district in Bitlis province. The analysis of variance indicated that the cultivar had a major influence on all parameters under evaluation ($p < 0.05$).

pH, total soluble solids and titratable acidity in black mulberry cultivars

pH value in black mulberry cultivars was between 3.65 (13-BIT-2) and 4.12 (13-BIT-5). The average pH of cultivars was 3.85. The pH

contents in different mulberries ranged from 3.3 to 3.8 (Uzun ve Bayir, 2009) and our pH results are generally within limits of these studies.

Total soluble solid content (TSS) in black mulberry cultivars varied from 15.65 (13-BIT-2) to 22.10 % (13-BIT-6) with an average of 18.94 %. Previous studies had shown that soluble solid content of mulberry fruits grown in different agroclimatic regions of Turkey is between 15.27–30.80 % (Lale and Ozcagiran, 1996; Aslan, 1988), and our SSC results are generally within limits of these studies.

The titratable acidity of black mulberry cultivars was between 1.45 % (13-BIT-1) and 1.85 % (13-BIT-4).

The average titratable acidity of black mulberry cultivars was 1.63 %, which is a little higher than those reported for red and white mulberries (Ercisli et al., 2010) and black mulberry (Iqbal et al., 2010).

Table 1. pH, TSS (%) and titratable acidity composition of black mulberry cultivars

Cultivars	pH	TSS (%)	TA
13-BIT-1	3.82 b	17.20 bc	1.45 d
13-BIT-2	3.65 c	22.10 a	1.75 b
13-BIT-3	3.90 ab	16.70 c	1.56 c
13-BIT-4	3.70 bc	20.50 a	1.85 a
13-BIT-5	4.12 a	18.40 b	1.62 bc
13-BIT-6	4.06 a	15.65 d	1.80 a
13-BIT-7	3.92ab	21.40 a	1.55 c
13-BIT-8	3.66 c	19.55 a	1.48 c
Mean value	3.85	18.94	1.63

Vitamin C, antioxidant activity, total phenolic and total anthocyanins content in black mulberry fruits

We found vitamin C content between 18.40 (13-BIT-3) and 23.67 (13-BIT-6) mg/100 g for black mulberry cultivars under the investigation. In the earlier work conducted on the northeast Anatolia region of Turkey, Ercisli and Orhan (2008) reported that vitamin C contents of black mulberry cultivars varied from 14.9 to 18.8 mg/100 mL. Ercisli et al. (2010) reported the average vitamin C content in black and purple mulberries as 20.79 and 18.87 mg per 100 mL extract, respectively. Lale and Ozcagiran (1996) reported that vitamin C content in black and purple mulberries was 16.6 and 11.9 mg/100 ml extract.

Table 2. Vitamin C, DPPH, total phenolic and anthocyanins content of black mulberry cultivars

Cultivars	Vitamin C	DPPH %	Total phenolic content	Total anthocyanins content
13-BIT-1	22.65 a	18.24	2125 c	815 a
13-BIT-2	19.50 b	21.56	1920 c	793 ab
13-BIT-3	18.40 bc	20.44	2330 ab	710 c
13-BIT-4	20.28 ab	19.86	2255 ab	826 a
13-BIT-5	21.15 b	22.38	1970 d	674 d
13-BIT-6	23.67 a	23.18	2345 ab	808 a
13-BIT-7	18.87 c	20.42	2575 a	756 b
13-BIT-8	22.36 b	18.66	2215 b	643 d
Mean value	20.86	20.59	2217	753.13

The antiradical activity of black mulberry cultivars were 18.24 (13-BIT-1) to 23.18 % (13-BIT-6) (DPPH assay).

Ozkaya (2015) reported that antioxidant activity in black mulberry were 15.037-24.443 μM TE/g. The results for total phenolics content between 1920 (13-BIT-2) and 2575 (13-BIT-7) GAE mg/g. Earlier reports had shown that the total phenolic content in mulberry fruits was between 1515–2570 GAE mg/g (Lin and Tang, 2007; Bae and Suh, 2007*)*. The difference between mulberry cultivars and between species in terms of phenolics is supposed to be a genetic characteristic because all plants were grown under the same agroclimatic conditions.

The effect of cultivar within the same fruit species on total phenolic content is well documented by several researchers on apples and strawberries (Scalzo et al., 2005; Voča et al., 2008), sea buckthorns (Ercisli et al., 2007) and cornelian cherries (Yilmaz et al., 2009). The total anthocyanin content per fresh weight of black mulberry (*Morus nigra*) cultivars ranged from 643 (13-BIT-4) and 826 (13-BIT-8) Cy 3-glu mg/g. According to earlier reports, total anthocyanin content in purple and black mulberries was 99 and 571 Cy 3-glu mg/g (Ozgen et al., 2009).

CONCLUSIONS

The results clearly indicate the difference between the cultivars used grown in the same conditions. Antioxidant activity also varies among the different cultivars of black mulberry, and this is a reflection of the phytochemical differences between cultivars.
These local black mulberry cultivars have high vitamin C, total phenolic, anthocyanin and antioxidant capacity in fruit. It is known, positive effect on human health of these substances.
This cultivars can be used for future breeding activities to obtain more healthier black mulberry.

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