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Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits

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Abstract

In this study, the chemical composition of white (*Morus alba* L.), red (*Morus rubra* L.) and black (*Morus nigra* L.) mulberry fruits grown in the East Anatolia Region of Turkey was investigated. The highest total phenolic and flavonoid contents were observed in black mulberry (1422 mg gallic acid equivalents/100 g fresh matter and 276 mg quercetin equivalents/100 g fresh matter). *M. alba* had the highest total fat content (1.10%), followed by *M. nigra* (0.95%) and *M. rubra* (0.85%), respectively. The major fatty acids in mulberry fruits were linoleic acid (54.2%), palmitic acid (19.8%) and oleic acid (8.41%), respectively. The total soluble solids content of mulberry species varied between 15.9% (*M. rubra* L.) and 20.4% (*M. alba* L.), acidity between 0.25% (*M. alba* L.) and 1.40% (*M. nigra* L.), pH between 3.52 (*M. nigra* L.) and 5.60 (*M. alba* L.), ascorbic acid 19.4 mg/100 g (*M. rubra* L.) and 22.4 mg/100 g (*M. alba* L.), respectively. Mineral compositions of the mulberry species were 0.83% N, 235 mg/100 g P, 1141 mg/100 g K, 139 mg/100 g Ca, 109 mg/100 g Mg, 60 mg/100 g Na, 4.3 mg/100 g Fe, 0.4 mg/100 g Cu, 4.0 mg/100 g Mn and 3.1 mg/100 g Zn, respectively.

Keywords: Mulberry species; Minerals; Fatty acids; Phenolics; Flavonoids

1. Introduction

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 is found from temperate to subtropical regions of the Northern hemisphere to the tropics of the Southern hemisphere and they can grow in a wide range of climatic, topographical and soil conditions. These are widely spread throughout all regions from the tropics to the sub-arctic and from sea level to altitudes as high as 4000 m (Machii, Koyama, & Yamanouchi, 2000; Tutin et al., 1996).

In most mulberry-growing countries, in particular in India and China, mulberry is used for its foliage, to feed the silkworm (*Bombyx mori* L.). Mulberry breeding has focused on enhancing foliage production in these countries (Vijayan, Chauhan, Das, Chakraborti, & Roy, 1997). However, in most European countries, including Turkey and Greece, mulberries are grown for fruit production rather than foliage (Ercisli, 2004; Gerasopoulos & Stavroulakis, 1997).

The Anatolia region of Turkey has growing conditions suitable for cultivating high quality mulberry fruits, mainly *Morus alba*, *Morus nigra* and *Morus rubra* (Yaltirik, 1982). The production of mulberry in Turkey in 2005 was 78,000 tonnes (Anonymous, 2005) and its cultivation in Turkey has been known for more than 400 years. 95% of the mulberry trees grown in Turkey are *M. alba*, 3% are *M. rubra* and 2% are *M. nigra* (Ercisli, 2004).

In Turkey, traditional products such as 'mulberry pekmez', 'mulberry pestil' and 'mulberry kome' are made with the fruits. The red-coloured fruits are eaten fresh and are also used in marmalades, juices, liquors, natural dyes and

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in the cosmetics industry (Ercisli & Orhan, 2005; Sengul, Ertugay, & Sengul, 2005). The plant has also been used medicinally in Turkey. Local people traditionally believe that deep-coloured fruits, especially black and red mulberry fruits, are healthier for the human body (Ercisli & Orhan, 2005). Mulberry fruits can be used as a worming agent, as a remedy for dysentery, and as a laxative, odontalgic, anthelmintic, expectorant, hypoglycaemic and emetic (Baytop, 1996).

Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic and anticarcinogenic properties, as well as the ability to modify gene expression (Nakamura, Watanabe, Miyake, Kohno, & Osawa, 2003; Tapiero, Tew, Ba, & Mathe, 2002). Deep-coloured fruits are good sources of phenolics, including flavonoids, anthocyanins and carotenoids (Cieslik, Greda, & Adamus, 2006; Sass-Kiss, Kiss, Milotay, Kerek, & Toth-Markus, 2005; Qian, Liu, & Huang, 2004; Trappey, Bawadi, Bansode, & Losso, 2005), and mulberries are rich in phenolics (Lin & Tang, 2007).

Fruit contains essential fatty acids that humans cannot synthesise, and must be obtained through diet. Essential fatty acids are long chain polyunsaturated fatty acids derived from linolenic, linoleic, and oleic acids, and they are necessary for the formation of healthy cell membranes, the proper development and functioning of the brain and nervous system, and for the production of hormone-like substances called eicosanoids (thromboxanes, leukotrienes, prostaglandins). These chemicals regulate numerous body functions including blood pressure, blood viscosity, immune and inflammatory responses (Pawlosky, Ward, & Salem, 1996; Simopoulos & Salem, 1996).

There have been studies on flavour, dry matter, total sugar, total acidity, ash, ascorbic acid, pH and anthocyanin content of some mulberry species (Elmaci & Altug, 2002; Gerasopoulos & Stavroulakis, 1997; Lale & Ozcagiran, 1996; Ozdemir & Topuz, 1998). However, the minerals content of mulberry fruits have not yet been investigated. Moreover, to our knowledge, there have been no comparative studies on the chemical composition of three mulberry species, namely *M. alba*, *M. nigra* and *M. rubra*, grown under the same ecological conditions. Therefore the aim of this study is to compare white, red and black mulberry species, in terms of chemical composition.

2. Materials and Methods

2.1. Collection and preparation of mulberry fruit samples

Mulberry fruits were harvested from selected *M. rubra* clone kirmizi, *M. nigra* clone siyah and *M. alba* clone pirinc from Olur town, Erzurum, Turkey, in 2005. Each clone includes 5 trees of the same age. All berries were picked at the commercially ripe stage. The berries were selected according to uniformity of shape and colour. The fruits were then stored in polyethylene bags at −20 °C (up to 1 month) until analysis.

2.2. Determination of fruit colour, weight, moisture, total soluble solids, total dry weight, pH and acidity of mulberry fruits

Fifty fruits from each clone (10 fruits per tree) of each species were used for analysis. All analyses were performed on these 50 samples. Skin colour of mulberry fruits was measured using a CR-400 colorimeter (Konica Minolta, Ramsey, NJ) and measurements recorded as *L*, *a* and *b* values. Colour values for each fruit were computed as means of three measurements taken from opposite sides at the equatorial region of the fruit (Ertekin, Gozlekci, Kabas, Sonmez, & Akinci, 2006). Fruit weight was measured by using a digital balance with a sensitivity of 0.001 g (Scaltec SPB31). The moisture of samples was determined by drying at 103 ± 2 °C until they reached constant weight (AOAC, 1984). Total dry matter of the fruits was determined according to the methods of AOAC (1984). Total soluble solid contents (TSS) were determined by extracting and mixing one drop of juice from each fruit into a digital refractometer (Model RA-250HE, Kyoto Electronics Manufacturing Co. Ltd., Japan,) at 22 °C. The pH measurements were made using a digital pH meter (WTW Inolab Level 1, Germany) calibrated with pH 4 and 7 buffers. Titratable acidity (TAc) was measured by the titrimetric method (AOAC, 1984). Titratable acidity of mulberry was expressed as % citric acid. TSS/TAc results are also determined.

2.3. Determination of total fat and fatty acid content

Total fat was extracted with *n*-hexane (60 °C) for 6 h using a Soxhlet extractor and fatty acid composition was analysed according to a previous method (Anonymous, 2000). Fatty acids were designated (e.g., 18:1 ω9c) so that the figures represent, from left to right, the total number of carbon atoms, the number of double bonds, the position of the double bond from the ω end of the fatty acyl chain and the configuration of the double bond.

2.4. Determination of ascorbic acid, total phenolic and flavonoid contents in mulberry fruits

Vitamin C content of the fruits was determined according to the methods of AOAC (1984). Total phenolic contents of mulberry fruits were determined by the Folin–Ciocalteu method (Meda, Lamien, Romito, Millogo, & Nacoulma, 2005). Briefly, aliquots of 0.1 g lyophilised powder of fruit samples were dissolved in 1 ml deionised water. This solution (0.1 ml) was mixed with 2.8 ml of deionised water, 2 ml of 2% sodium carbonate (Na_2CO_3), and 0.1 ml of 50% Folin–Ciocalteau reagent. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 750 nm against a deionised water blank. Gallic acid (GA) was chosen as a standard. Using a seven point standard curve (0–200 mg/l), the total phenolic contents in mulberry

fruits were determined. The data were expressed as milligram gallic acid equivalents (GAE) per gram of lyophilised powder. Finally, the data were converted to mg GAE/100 g fresh matter of fruit, based on the moisture contents of lyophilised powder and fresh fruit material. The total flavonoid content of mulberry fruits was determined using the aluminum chloride colorimetric method described by Chang, Yang, Wen, and Chern (2002). Briefly, aliquots of 0.1 g of fruit samples were dissolved in 1 ml deionised water. This solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionised water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against a deionised water blank. Quercetin was chosen as a standard. Using a seven point standard curve (0–50 mg/l), the levels of total flavonoid contents in mulberry fruits were determined. The data were expressed as milligram quercetin equivalents (QE) per gram of lyophilised powder. The data were then converted into mg QE/100 g fresh matter, based on the moisture content of lyophilised powder and fresh fruit.

2.5. Determination of mineral content

Total N was determined by the micro-Kjeldahl method (James, 1995). In order to determine the mineral composition, samples were burned with a nitric acid/perchloric acid solution, on a hot plate, at 200 °C. Then, the absorbance of the extract was measured by atomic absorbance spectroscopy. The amounts of minerals were calculated with a standard curve of each element. Phosphorus content of the extract, however, was analysed by determining the yellow absorbance, obtained from the Barton reaction, at 680 nm, and comparing the results to a standard curve (James, 1995).

2.6. Statistical analysis

One way analysis of variance (ANOVA), with multiple range significant difference (LSD) test ($p < 0.05$) were carried out using SPSS.

3. Results and discussion

3.1. Fruit colour, weight, moisture, total soluble solids, total dry weight, pH and acidity in mulberry fruits

The fruit weight, fruit colour, moisture, pH, acidity, TSS (Total soluble solids) and TDW (Total dry weight) contents of mulberry species are given in Table 1. Fruit weight of mulberry species ranged 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*). According to the results, *M. alba* fruits may be recommended for processing, due to higher TSS and TDW contents, and *M. nigra* may be recommended for fresh fruit production, since it has attractive bigger fruits. High fruit TSS and acid content, is a combination found only in a few other fruits, such as pomegranate and kiwi fruit.

Fruit weight, TSS, pH and acidity of fruit of *M. alba*, *M. rubra* and *M. nigra*, grown in a different region of Turkey were 0.90–3.82 g, 15.27–30.80%, 3.60–5.65, and 0.17–2.40%, respectively (Aslan, 1998; Cam, 2000; Lale & Ozcagiran, 1996; Ozdemir & Topuz, 1998). Our fruit weight, TSS, pH and acidity results in general were within the limits of these studies. The variation of fruit weight, TSS, pH and acidity in mulberry fruits could be due to different species, cultivars, rootstocks used, environmental conditions and the nutritional status of the orchards.

3.2. Total fat and fatty acid composition of mulberry fruits

The total fat contents of the mulberry species were low and were between 0.85% (*M. rubra*) and 1.10% (*M. alba*), respectively. *M. alba* which had the lowest moisture content had the highest total fat (1.10%), followed by *M. nigra* (0.95%) and *M. rubra* (0.85%).

Fatty acid analysis has shown that the mulberry species studied contained fourteen major compounds and significant variation of fatty acids was found between species (Table 2). Linoleic acid, C18:2, was the dominant fatty acid (43.4–61.9%) in all mulberry species, followed by palmitic acid C16:0 (12.1–24.8%) (Table 2). Our results are in agreement with Elmaci and Altug (2002), who reported that the fatty acids found at the highest levels in fruits of three black mulberry (*M. nigra*) cultivars were linoleic acid (13.6–44.4%), palmitic acid (14.4–22.7%) and oleic acid (2.33–16.0%).

Total peak areas of the mentioned fatty acids were 90.7% in *M. rubra*, 98.1% in *M. alba* and 97.9% in *M. nigra*, respectively. Behenic (C22:0) and palmitoleic (C16:1) acids were detected only in *M. alba* fruits (0.26% and 0.67%, respectively). *M. rubra* was the only species that contained *cis*-C18:3 ω3 and *cis*-C19:1 ω7 at 8.50% and 1.09%, respectively. Oleic acid (C18:1) was only found in *M. nigra* (14.8%) and *M. alba* (10.5%). All species contained myristic (C14:0) (0.98–2.51%) and stearic (C18:0) acids (4.27–6.19%), (Table 2). According to literature searched, this is the first study on the fatty acid content of mulberry species. Our results suggest that fatty acid profile can be useful for identification of mulberry species.

Table 1
Fruit weight, colour, moisture, pH, TAc (total acidity), TSS (total soluble solids), TDW (total dry weight) and TSS/TAc contents of mulberry species

Species	Fruit weight (g)	Fruit colour			Moisture (%)	pH	TAc (%)	TSS (%)	TDW (%)	TSS/TAc
		L	*a*	*b*						
Morus alba	3.49b	78.4a	−13.6b	16.2a	71.5	5.60a	0.25b	20.4a	29.5a	81.6a
Morus nigra	4.37a	14.3c	7.02a	1.72b	72.6	3.52c	1.40a	16.7b	27.4ab	12.0b
Morus rubra	2.14c	27.3b	8.55a	2.02b	74.6	4.04b	1.37a	15.9b	24.4b	11.6b

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

Table 2
Total fat (%) and fatty acid content (%) of mulberry species

Fatty acid	Fatty acid content (%)		
	Morus alba	*Morus nigra*	*Morus rubra*
14:0	0.98b	1.10b	2.51a
16:0	22.42a	12.06b	24.79a
cis-C16:1 ω7	0.67a	0.00b	0.00b
18:0	4.27a	5.86ab	6.19b
cis-C18:1 ω9	10.49b	14.75a	0.00c
cis-C18:2 ω6	57.26b	61.85a	43.39c
cis-C18:3 ω3	0.00b	0.00b	8.50a
cis-C19:1 ω6	0.62c	1.35b	3.36a
cis-C19:1 ω7	0.00b	0.00b	1.09a
22:0	0.26a	0.00b	0.00b
Total fat (%)	1.10a	0.95ab	0.85b

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

3.3. Ascorbic acid, total phenolic and flavonoid contents in mulberry fruits

The ascorbic acid, total phenolic and total flavonoid content of mulberry species are given in Table 3. Ascorbic acid contents of the mulberry species were found to be from 19.4 mg/100 ml (*M. rubra*) to 22.4 mg/100 ml (*M. alba*), (Table 3). In a previous study conducted in the Aegean region of Turkey, the highest ascorbic acid content was found in *M. alba* (17.8 mg/100 ml) and followed by *M. nigra* (16.6 mg/100 ml) and *M. rubra* (11.9 mg/100 ml) (Lale & Ozcagiran, 1996).

Mulberries are a rich source of phenolics, with high levels in *M. nigra* (1422 mg GAE/100 g) and *M. rubra* (1035 mg GAE/100 g). *M. nigra* and *M. rubra* are also high in flavonoids (276 and 219 mg QE/100 g fresh mass, respectively). Differences in terms of phenolics and flavonoids content are due to genetic derivation because all plants were of the same age and grown under the same ecological conditions. It is reported that plant genotype (Scalzo, Politi, Pellegrini, Mezzetti, & Battino, 2005) and cultivation (Hakkinen & Torronen, 2000) affect total phenolic and flavonoid contents in fruit. The colour substances tend to concentrate at the outer druplet cells in *M. alba*, whereas in the *M. nigra* and *M. rubra* they concentrate in all the cells of drupelets. In Turkey, in particular Eastern Anatolia region, local peoples use *M. nigra* fruits as medicine. Our phenolics and flavonoid results of this species support this idea. It is clear that phenolic acids levels in red and black mulberries explain their sour, astringent taste. The variation of phenolic compounds in the fruits depends on many factors, such as degree of maturity at harvest, genetic differences, and environmental conditions during fruit development, etc. (Zadernowski, Naczk, & Nesterowicz, 2005). In red-coloured fruits phenolics increase during the last ripening stage, due to the maximal accumulation of anthocyanins and flavonols (Bridle & Timberlake, 1978; Gerasopoulos & Stavroulakis, 1997).

Table 3
Ascorbic acid, total phenolic and total flavonoid content of mulberry species

Species	Total phenolics (mg GAE/100 g fresh mass)	Total flavonoids (mg QE/100 g fresh mass)	Flavonoids/Phenolics	Ascorbic acid (mg/100 ml)
Morus alba	181c	29c	0.16	22.4a
Morus nigra	1422a	276a	0.19	21.8ab
Morus rubra	1035b	219b	0.21	19.4b

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

3.4. Mineral elements in mulberry fruits

The mineral contents of mulberry species are shown in Table 4. Differences among the mulberry species were observed based on the mineral compositions. The N, P, K and Ca values of mulberry species varied from 0.75% (*M. alba*) to 0.92% (*M. nigra*), 226 mg/100 g (*M. rubra*) to 247 mg/100 g (*M. alba*), 834 mg/100 g (*M. rubra*) to 1668 mg/100 g (*M. alba*), 132 mg/100 g (*M. nigra*, *M. rubra*) to 152 mg/100 g (*M. alba*), and 106 mg/100 g (*M. alba*, *M. nigra*) to 115 mg/100 g (*M. rubra*), respectively (Table 4). The mineral composition of fruits depended, not only on the species or varieties, but also on the growing conditions, such as soil and geographical conditions. In this study, while the existence of ten elements was determined in all mulberry species, K was predominant, followed by N, P, Ca, Mg, Na, Fe, Mn, Zn and Cu.

As a conclusion of this study, it can be said that mulberry fruits are a valuable horticultural product, based on their rich and beneficial nutrient composition. Certain growing conditions and cultural management techniques, affecting the nutritional value of mulberry species, will be the subject of further research projects.

Table 4
Mineral contents of mulberry species

Species	Mineral elements (mg/100 g)									
	N (%)	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Morus alba	0.75c	247a	1668a	152a	106b	60	4.2b	0.5	3.8b	2.8b
Morus nigra	0.92a	232ab	922b	132b	106b	59	4.2b	0.4	4.2a	3.2a
Morus rubra	0.82b	226b	834c	132b	115a	61	4.5a	0.4	4.0ab	3.2a

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

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