ISSN 1330-9862 *scientific note*
(FTB-2241)

Phytochemical Content of Some Black (*Morus nigra* L.) and Purple (*Morus rubra* L.) Mulberry Genotypes

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Received: February 23, 2009
Accepted: July 9, 2009

Summary

Bright black (*Morus nigra*) and purple mulberry (*Morus rubra*) are particularly desirable fruits in Turkey. More recently, the interest in these bright black and purple mulberry fruits has also increased because of the popularization of healthy properties of these fruits. The study was carried out in 2008 aiming to determine the antioxidant activity (ferric reducing ability of plasma, FRAP), total phenolic, total anthocyanin, mineral, soluble solid, vitamin C, and total acid content of four black and four purple mulberry genotypes grown in Turkey. The results show that black mulberry genotypes have a higher bioactive content than purple mulberry genotypes. The average total phenolic content and total anthocyanins of black mulberry genotypes were 2149 μg of gallic acid equivalent (GAE) per g and 719 μg of cyanidin 3-glucoside equivalent (Cy 3-glu) per g of fresh mass. In purple mulberry, these values were for GAE 1690 μg/g and for Cy 3-glu 109 μg/g on fresh mass basis. The average antioxidant activity of black mulberry genotypes was also found to be higher than that of the purple ones according to FRAP assay (Trolox equivalent (TE) per fresh mass of black and purple mulberries was 13.35 and 6.87 μmol/g, respectively).

Key words: black and purple mulberry, antioxidant activity, mineral elements

Introduction

There is a great interest in determining the role of phytonutrients in promoting better health and to reduce cancer, cardiovascular diseases and the effects of aging. It is widely believed that antioxidant phytonutrients can inhibit the propagation of free radical reactions that may ultimately lead to the development of diseases, especially those which are ageing-related. Analysis in several laboratories shows that many fruits and vegetables have strong antioxidant capacities, and that this capacity is due primarily to non-vitamin C phytochemicals (*1,2*).

Dark-coloured fruits, particularly berries (strawberry, raspberry, blackberry, blueberry, mulberry, *etc.*) are recognized as being healthy. 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 (*3*).

The black and purple mulberries are widely cultivated in the Mediterranean and Middle Eastern countries (*4*). Located at the junction of the Middle East and the Mediterranean, Turkey has important black and purple mulberry populations and the cultivation of these fruits has been known for more than 400 years (*5*). The

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bright black and purple mulberry fruits, which have a very pleasant taste when eaten fresh, are also used in jams, juices, liquors, natural dyes as well as in the cosmetics industry (*6*).

Black and purple mulberry fruits have also been effectively used in folk medicine in Turkey for a long time to treat fever, protect liver from damage, strengthen the joints, facilitate discharge of urine and lower blood pressure (*7*). Recently, black and purple mulberries have gained an important position in the local soft drink market, although their biological and pharmacological effects are still poorly defined. These fruits are also widely consumed by the inhabitants of the rural parts of Turkey. Therefore, the focus of the present study is on phytochemical components and antioxidant activity of four black and four purple mulberry genotypes.

Materials and Methods

Collection and preparation of black and purple mulberry fruit samples

A total of eight mulberry genotypes, four black (*Morus nigra* L.) and four purple mulberry (*Morus rubra* L.) species grown in Coruh Valley were used in the present study. Approximately 1 kg of fresh fruits per genotype were collected at peak ripeness and quickly transported to the laboratory at Ataturk University, where fruit samples were analyzed immediately. The fruits were mashed in a homogenizer and prepared for further analyses. Four replicates were used per analysis. The parameters analyzed were: soluble solid content (SSC), total acidity, ascorbic acid (AsA), total phenolics (TP), total anthocyanins (TA) and antioxidant capacity (AC).

Determination of total soluble solids, total acidity and ascorbic acid in black and purple mulberry fruits

A total of 30 fruits were taken for each replication on which analyses were performed. Soluble solid content expressed as percentage was determined by a digital refractometer (Model RA-250HE Kyoto Electronics, Kyoto, Japan). Total acidity was determined by AOAC method (*8*) and expressed as percentage. Ascorbic acid was quantified with the reflectometer (Reflectometer RQflex®, Merck KGaA, Darmstadt, Germany). Results were expressed as mg of ascorbic acid (AsA) per 100 mL.

Determination of total anthocyanins, total phenolic content and total antioxidant capacity in mulberry fruits

Before extraction, fresh black and purple mulberry fruits were homogenized in a house blender and analytical determinations were carried out on fruit homogenates. The homogenates were used to evaluate the total anthocyanins (TA), total phenols (TP) and total antioxidant capacity (TAC). The TA content was determined using bisulphate bleaching method (*9*). Results were expressed as µg of cyanidin 3-glucoside equivalent per g of fresh mass. TP content was estimated using the Folin-Ciocalteu colorimetric method described by Ough and Amerine (*10*). Concentration of TP was expressed as µg of gallic acid equivalent (GAE) per g of fresh mass. TAC was estimated by using ferric reducing ability of plasma (FRAP) (*11*) and the 2,2-diphenyl-1-picrylhydrazil (DPPH) assays to measure the free radical scavenging capacity of fruit extracts (*12*). Results were expressed in µmol of Trolox equivalents per g of fresh mass for both FRAP and DPPH assays.

Determination of mineral elements

Fruit samples were oven-dried at 68 °C for 48 h and ground to pass through 1-mm sieve. The Kjeldahl method (*13*) and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Königswinter, Germany) were used to determine total nitrogen. Phosphorus content was determined after wet digestion using an HNO_3-$HClO_4$ acid mixture (4:1 by volume) (*8*). Phosphorus in the extraction solution was measured spectrophotometrically using the indophenol-blue and ascorbic acid method with a UV/VIS Aqumat Spectrophotometer (Thermo Electron Spectroscopy Ltd, Cambridge, UK). K, Ca, Mg, Fe, Mn, Zn and Cu were determined after wet digestion using an HNO_3-$HClO_4$ acid mixture (4:1 by volume) with a PerkinElmer 360 Atomic Absorption Spectrophotometer (PerkinElmer, Waltham, MA, USA). Results for the minerals (N, P, K, Ca, Mg, Na, Fe, Zn and Mn) were expressed in mg per 100 g of fresh mass.

Statistical analysis

The experiment was a completely randomized design with four replications. Data were subjected to analysis of variance (ANOVA) and means were separated by Duncan's multiple range test at $p<0.05$ significance level. SAS procedure was used as a statistical program.

Results and Discussion

Soluble solid content, vitamin C and total acidity in the black and purple mulberry fruits

The SSC, vitamin C and total acidity of black and purple mulberry genotypes are shown in Table 1, where statistical differences in the amounts of these components, both within purple and black mulberry genotypes can also be seen.

SSC in black mulberry genotypes varied from 16.95 (MN3) to 18.40 % (MN1) with an average of 17.63 %, while in purple mulberry genotypes it ranged from 14.38 (MR3) to 15.11 % (MR1) with an average of 14.87 %, indicating lower average values than in the black mulberry. Previous studies had shown that purple mulberry had lower soluble solid content than black mulberry (*14,15*). Soluble solid content of mulberry fruits grown in different agroclimatic regions of Turkey is between 15.27–30.80 % (*16,17*), and our SSC results are generally within limits of these studies.

The average vitamin C content in black and purple mulberries was 20.79 and 18.87 mg per 100 mL, respectively (Table 1). Fruit species can be classified into three groups (low, moderate and high) in terms of their vitamin C content (*18*) and mulberries are generally placed within the moderate vitamin C content group. Lale and Ozcagiran (*16*) reported that vitamin C content in black

Table 1. Phytochemical composition of black and purple mulberry genotypes

Species	Genotypes	*w*(SSC) / %	*γ*(vitamin C) / mg/100 mL	Total acidity / %	*w*(total anthocyanin as Cy 3-glu) / μg/g	*w*(total phenolics as GAE) / μg/g	*w*(FRAP as TE) / μmol/g	*w*(DPPH as TE) / μmol/g
Morus nigra	MN1	18.40a	22.60a	1.64ab	787a	2483a	14.11a	21.17
	MN2	17.10b	18.10b	1.93ab	674c	1826d	12.26d	16.22
	MN3	16.95b	21.35ab	1.97a	723b	2200b	13.94b	19.87
	MN4	18.05a	21.10ab	1.81b	693c	2087c	13.10c	18.64
Mean value		17.63	20.79	1.84	719	2149	13.35	18.98
Morus rubra	MR1	15.11a	19.02ab	1.04b	81c	1584d	4.93d	9.22
	MR2	14.97ab	18.71ab	1.10ab	118a	1713b	7.61b	12.06
	MR3	14.38b	18.12b	0.96ab	106b	1674c	6.82c	11.41
	MR4	15.02ab	19.64a	1.07ab	132a	1789a	8.12a	12.15
Mean value		14.87	18.87	1.06	109	1690	6.87	11.21

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

and purple mulberries was 16.6 and 11.9 mg/100 mL, which is in accordance with our results.

The total acidity of black mulberry genotypes was between 1.64 and 1.97 %, whereas these values were between 0.96 and 1.10 % in purple mulberry genotypes. The average total acidity of black mulberries was 1.84, and of purple mulberries 1.06 %. It can be said that purple mulberries have lower acidity compared to black mulberries. However, when considering SSC and acidity together, it can be said that black mulberry may be recommended for fresh fruit production since it has attractive large fruits and combines high fruit SSC and acid content, which gives them a pleasant taste.

Total anthocyanins, total phenolic content and total antioxidant capacity in black and purple mulberry fruits

The differences in total anthocyanins, total phenolic content and total antioxidant capacity among different genotypes within the same species were found to be statistically significant ($p<0.05$, Table 1). The total anthocyanin content per fresh mass of black mulberry (*Morus nigra*) genotypes ranged from 674 (MN2) to 787 (MN1) Cy 3-glu μg/g, and from 81 (MR1) to 132 (MR4) Cy 3-glu μg/g for purple (*Morus rubra*) genotypes (Table 1). The results also showed that average anthocyanin content of black mulberries was 7 times higher than that of purple mulberries. The genotype seems to affect the total anthocyanin content in the berries. According to earlier reports, total anthocyanin content in purple and black mulberries was 99 and 571 Cy 3-glu μg/g (*15*).

The results for total phenolics clearly showed that fruits of black mulberry (*Morus nigra*) (1826–2483 GAE μg/g) had a higher total phenolic content than those of the purple mulberry (*Morus rubra*) (1584–1789 GAE μg/g), see Table 1. Earlier reports had shown that the total phenolic content in mulberry fruits was between 1515–2570 GAE μg/g (*3,19*). The difference between mulberry genotypes 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 genotype within the same fruit species on total phenolic content is well documented by several researchers on apples and strawberries (*20,21*), sea buckthorns (*22*) and cornelian cherries (*23*).

The antioxidant activity (FRAP and DPPH assays) in berries of different black and purple mulberry genotypes is shown in Table 1. A statistically significant difference ($p<0.05$) was found between the samples with both methods used. In FRAP assay, it was found that black mulberry genotypes had the average antioxidant activity of 13.35 and purple mulberry genotypes of 6.87 TE μmol/g. The order of antioxidant capacities expressed as TE μmol/g in FRAP assay within black and purple mulberry genotypes was MN1 (14.11)>MN3 (13.94)> MN4 (13.10)>MN2 (12.26)>MR4 (8.12)>MR2 (7.61)>MR3 (6.82)>MR1 (4.93) (Table 1). The FRAP value of antioxidant-rich berry fruits was 82.3 in bilberry, 73.5 in blackcurrant, 43.1 in elderberry, 50.7 in blackberry, 21.7 in strawberry, 17.8 in red currant, 14.5 in gooseberries and 2.0–26.5 TE μmol/g in vegetables including pepper, kale, parsley, spinach, celery, onion, radish, lettuce, tomato, garlic, cucumber and squash (*24*). It can be said that mulberries are richer in antioxidants than vegetables, and close to gooseberries and red currant.

In DPPH assay, the genotypes belonging to black and purple mulberry genotypes revealed parallel trend to FRAP assay. The order of antioxidant capacities expressed as TE μmol/g in DPPH assay within black and purple mulberry genotypes was MN1 (21.17)>MN3 (19.87)>MN4 (18.64)>MN2 (16.22)>MR4 (12.15)>MR2 (12.06)>MR3 (11.41)>MR1 (9.22) (Table 1). These results indicate that black mulberries had higher antioxidant capacity than purple mulberries. In fact, in recent years, the number of black mulberry orchards has increased in Turkey because of higher demand by consumers. In contrast, the number of purple mulberry trees decreased. According to these results, it can be said that mulberries have moderate antioxidant activity among berry fruits. Previous studies on mulberries also concluded that mulberry fruits had moderate antioxidant activity (*3*), which supports our findings.

Mineral content of black and purple mulberry fruits

The mineral content of black and purple mulberry genotypes is shown in Table 2. The statistical differences between the genotypes were observed based on N, P and K contents (Table 2). The average N, P and K values per 100 g in black mulberry genotypes were 800, 289 and 1005 mg, respectively, while in purple mulberry genotypes they were 690, 242 and 929 mg, respectively (Table 2). Data obtained from black and purple mulberry genotypes show that they have very high nutritional potential, and their nutritional value is greater than that of some cultivated fruits presented in Table 3 (*25*). There is a growing interest in the mineral content of foods and diets. Experiments in cell culture and in intact organisms reveal the importance of macro and trace elements in many metabolic processes and functions throughout the life cycle. Human as well as animal studies have shown that optimal intake of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc, and iodine could reduce individual risk factors, including those related to cardiovascular diseases (*26*). As in most vegetarian diets, protein quality and quantity are major concerns. Most plants contain incomplete proteins, but combining different plant foods (nutrient supplementation) may improve the situation. Lack of adequate proteins, either in quality or quantity contributes to low body mass, growth retardation in children, and developmental deficiency during pregnancy. The average adult requires approx. 0.8 g of protein per kg of lean body mass per day to maintain normal functions, and so a person weighing 70 kg needs approx. 56 g of protein daily. To a certain extent, the use of black or purple mulberry genotypes in a diet may contribute to filling the protein gap. Calcium eases insomnia and helps regulate the passage of nutrients through cell walls. Iron deficiency in infants can result in impaired learning ability and behavioural problems. With respect to their Ca and Fe content, the mulberry genotypes considered in this study may offer a better nutritional potential. Potassium is essential for the body's growth and maintenance. Many studies suggest a relationship between high dietary K intake and lower blood pressure and protection from the risk of stroke (*27,28*). Similarly zinc, a trace mineral that is especially important for the normal functioning of the immune system, is relatively abundant in black and purple mulberry genotypes in comparison with some cultivated fruits.

Table 2. Mineral content of black and purple mulberry genotypes

Species	Genotype	*w*/(mg/100 g)								
		N	P	K	Ca	Mg	Na	Fe	Mn	Zn
Morus nigra	MN1	680b	314b	1314a	145^{ns}	114^{ns}	55^{ns}	5^{ns}	6^{ns}	3^{ns}
	MN2	910a	334a	922b	138	107	64	6	7	2
	MN3	880ab	291c	912b	135	111	60	5	6	4
	MN4	740ab	218d	873b	129	98	51	5	8	4
Mean value		800	289	1005	137	108	58	5	7	3
Morus rubra	MR1	710^{ns}	226bc	1118a	169a	97^{ns}	43^{ns}	5^{ns}	5^{ns}	4^{ns}
	MR2	620	243bc	773d	118b	89	47	4	5	4
	MR3	740	198c	961b	173a	93	51	5	5	3
	MR4	700	301a	862c	110b	84	39	4	6	2
Mean value		690	242	929	143	91	45	5	5	3

Values in the same column with different lower case letters are significantly different at $p<0.05$
^{ns}not significant

Table 3. Mineral content of some selected fruits compared to black and purple mulberries (*25*)

Fruits	*w*/(mg/100 g)						
	K	Ca	Mg	P	Fe	Mn	Zn
Morus nigra	1005	137	108	58	5	7	3
Morus rubra	929	143	91	45	5	5	3
Apple	158	9.5	7	9.5	*	*	*
Avocado	1204	22	78.4	82.4	2	*	*
Banana	467	7	43	27	0.4	*	*
Blackberries	282	46	28	30	0.8	1.9	0.4
Grapes	176	13	4.6	9	0.4	*	*
Kiwi	588	46	53	71	0.7	*	0.3
Mango	323	20.7	18.6	22.8	0.3	*	*
Orange	237	52	13	18	*	*	*
Peach	193	5.0	69	12	*	*	*

*Trace amount

Magnesium is needed for bones, proteins, making new cells, activating B vitamins, relaxing nerves and muscles, preventing blood clotting, and for energy production. Due to the high content of K, P and Mg, the black and purple mulberry genotypes could meet the daily K, P and Mg requirements of an adult.

Conclusion

The present study reveals that black and purple mulberry genotypes have relatively high nutritional potential and a wide variation was observed among genotypes in terms of nutrient contents. The data obtained from black and purple mulberry genotypes also show that their mineral content was greater than that of some other cultivated fruits. This could be important for breeding activity of mulberries. It can be concluded that black mulberry fruits are an inexpensive source of a number of nutrients, they provide macro- and microminerals and have a suitable taste and colour in diets used for human nutrition.

Acknowledgement

The authors are thankful for the partial support from COST 863 (TUBITAK-TOVAG 105-O-354).

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