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Antioxidant activity and antioxidants of raspberry and mulberry fruit

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Abstract

Mulberry (*Morus nigra* L.) and raspberry (*Rubus caesius* L.) are particularly desirable fruits in Iran. More recently, the interest in these mulberry and raspberry fruits has also increased because of the population of healthy properties of these fruits. The study was carried out in 2013 aiming to determine the antioxidant activing (2, 2-diphenyl-1-picrylhyrazyl, DPPH), total phenolic, total anthocyanin and ascorbic acid of mulberry and raspberry species grown in Iran. The results show that mulberry species has higher bioactive contents than raspberry species. The average total phenolic content and total anthocyanins of mulberry species was 345.77 mg GAE /100gFW and 75.7 mg/100g FW. In raspberry, these values were 242.97 mgGAE/100gFW and 50.14 mg /100gFW. The average antioxidant activity of mulberry species was also found to be higher than that of the raspberry ones according to DPPH assay.

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Introduction

In the last few years, there has been a growing interest in providing natural antioxidants. The protective effects of fruits and vegetables against coronary heart disease, stroke, and cancer have been attributed to the presence of flavonoids and other phytochemicals (Cacace and Mazza., 2002). All aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damages and repair enzymes to remove or repair damaged molecules. However, this natural antioxidant mechanism can be inefficient, and hence dietary intake of antioxidant compounds is important. Recent reports indicated that there is an inverse relationship between dietary intake of antioxidant rich foods and the incidence of human diseases (Odukoya *et al.*, 2005).

Black mulberry, *Morus nigra* L. (Moraceae), is a deciduous tree growing to a height of 10 to 13 m. The leaves are from 10 to 20 cm long. The tree yields dark purple_black, edible fruits that are 2-3 cm long after they have matured. The genus Morus is widespread in Asia, Europe, North and South America and Africa. Mulberry grows in the temperate and sub tropical regions of the northern hemisphere and it can grow in a wide range of climatic, topographic and soil conditions. Black mulberry has a unique delicious fruit, sour and refreshing taste. It has been used as a folk remedy to treat oral and dental diseases, diabetes, hypertension, arthritis and anemia (Ozgen *et al.*, 2009). Mulberry has high level of anthocyanins, hence it has a very important role in the food industry. The fruit color has been attributed to the anthocyanins present in the fruit. This has contributed to the positive effects of the fruit on people's health (Miliauskas *et al.*., 2004). Different researchers investigated certain properties of whole mulberry fruit, leaves, bark, root and extracts of part of mulberry trees. Few species of mulberry were evaluated for their edible fruits. Previously, some authors (Ozgen *et al.*, 2009; Ercisli and Orhan., 2008) studied the quality, nutritional potentials and chemical composition of some of the Morus species.

Raspberry fruits (Rubus sp) are highly appreciated by consumers for their aromatic taste. They provide nutrients and micronutrients essential for health, particular vitamin C and a significant dietary source of numerous phytochemicals with health benefits, mainly anthocyanins and phenolics (de Ancos *et al.*, 1990; Wang and Lin., 2000; Mullen *et al.*, 2002). It is a deciduous crop that belongs to complex of small fruit species called brambles which have perennial root system and biennial canes. The raspberry is an aggregate fruit, developed from an individual flower with several ovaries from which originate clusters of fleshy drupelets surrounding a hard-coated achene (Gabriel., 2006).

Raspberry and mulberry fruits are widely in some regions of Iran (Azerbaijan and Ardebil provinces). Despite its wide usage in this country, there have been no standardized studies on the fruits as the case is for other fruits species. The objective of this study was to determine antioxidant activity, total anthocyanins, total phenolic, ascorbic acid, and total flavonoids of a number of selected raspberry and mulberry fruits in Iran.

Materials and methods

Fruit materials

Raspberry (*Rubus. Caesius* L) and mulberry (*Morus nigra* L.) were collected from the northwest (Arasbaran- East Azerbaijan province) of Iran. The fruits were sorted according to uniformity of shape and color and then immediately transported to lab within 30 min after harvest and damage free were selected, immediately treated with liquid nitrogen and stored at -80˚C until extraction.

Ascorbic Acid (Vitamin C)

Total ascorbic acid content was determined using the dinitrophenylhydrazine (DNPH) method (Terada et al., 1978). Five grams of homogenized fruit tissue was added to 100 ml of a mixture of 6% metaphosphoric acid in 2 $moll^{-1}$ acetic acid. The mixture was centrifuged at 17,000 × g for 15 min at 4°C and supernatant was filtered through Whatman filter

paper. One milliliter aliquot of the supernatant was mixed with 0.05 ml of 0.2% 2, 6-dichlorophenolindolphenol (DCIP) and the solution was incubated at room temperature for 1 h. After that, 1 ml of 2% thiourea in 5% metaphosphoric acid and 0.5 ml of 2% DNPH in 4.5 $moll^{-1}$ sulfuric acid were added to the solution, and then incubated at 60°C for 3 h. The reaction was stopped by placing the tubes in an ice bath and slowly adding 2.5 ml of cold 90% sulfuric acid. Total ascorbic acid was measured by absorbance at 540 nm using a standard curve. The concentrations were expressed as ascorbic acid on a fresh weight basis, mg per 100 g of fruit.

Total anthocyanin content

Some of frozen tissue was ground to a fine powder under liquid nitrogen by cold mortar and pestle and 1g of the resultant powder was added to 10 ml of methanol containing HCl (1%, v/v) and held at 0°C for 10 min (Cordenunsi *et al.*, 2003). The slurry was centrifuged at 17,000× g for 15 min at 4 °C and then the supernatant was used. Total anthocyanins content was measured with the pH differential absorbance method, as described by Cheng and Breen (1991). Briefly, absorbance of the extracts were measured at 510 and 700 nm in buffers at pH 1.0 (hydrochloric acid-potassium chloride, 0.2 M) and 4.5 (acetate acid-sodium acetate, 1 M). Anthocyanin content was calculated using a molar extinction coefficient of 29,600 (cyaniding-3- glucoside).

$$\text{Absorbance (A)} = (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$$

Results were expressed as mg cyaniding 3-glucoside equivalent per 100g of fresh weight.

Total phenolic content

Total phenol in the methanol extracts was determined with Folin-Ciocalteu reagent by the method of Slinkard and Singleton (1972). Gallic acid (GAE) was used as a standard and results were expressed as mg gallic acid equivalents per 100 g fresh weight.

Total flavonoid content

Some of frozen tissue was ground to a fine powder under liquid nitrogen by cold mortar and pestle. One gram of the resultant powder was added to 10 ml of methanol containing HCl (1%, v/v) and held at room temperature for 24 h (Cordenunsi *et al.*, 2003). The slurry was centrifuged at 4000× g for 15 min at 4°C, and the supernatant was used. The total flavonoid contents were determined by a colorimetric assay (Yanping *et al.*, 2004). One milliliter aliquot of appropriately diluted sample was added to a 15 ml tube containing 4ml of deionized water. Then 0.3 ml of 5% $NaNO_2$ was added to this mixture, which was allowed to stand for 5 min at room temperature, and 0.6 ml of 10% $AlCl_3.6H_2O$ was added. The mixture was allowed to stand for 6 min at room temperature, and 2 ml of 1 mol l^{-1} NaOH was added, and the total was made up to 10 ml with deionized water. The absorbance of the solution was measured immediately at 510 nm. Quercetin was used as a standard compound for the quantification of total flavonoid

Antioxidant activity by DPPH radical scavenging method

The antioxidant capacity of the raspberry fruits were evaluated by free radical 2, 2-dipheynl-1-picrylhydrazyl (DPPH) methods. For the determination of free radical scavenging capacity, raspberry samples were extracted with methanol. Then, they were centrifuged (Sigma 3K30, Germany) at 15,000× g for 10 min. The supernatants were concentrated under reduced pressure at 40° C. The dried extracts were dissolved in methanol. Free radical scavenging activity was measured according to the principle of Nakajima *et al.* (2004) with some modifications reported by Chiou *et al.* (2007). Fifty microliters of the diluted extracts (concentrations 2-20 mg ml^{-1}) were added to 1 ml of 6× 10^{-5} mol l^{-1} DPPH (free radical, 95%, sigma-Aldrich Chemie GmbH, Steinheim, Germany) in methanol. The mixture was shacked and left at room temperature for 30 min; the absorbance was measured spectrophotometrically at 515 nm. Methanol was used

as an experimental control. The percent of reduction of DPPH was calculated according to the following equation

$$\%\,\text{inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sampele}}{\text{Abs control}} \times 100$$

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (SEM) of three determinations. Significant differences among mean values, where applicable, were determined by one_way analysis of variance (ANOVA), while differences among samples were determined by *Duncan's Multiple Range test using SPSS for Windows version 16.0 (SPSS Inc, USA).*

Results and discussion

Many reports have revealed that the physiological function of natural foods can be attributed to the antioxidative capacity of their phenolic components (Ness *et al.*, 1997; Halliwell *et al.*, 1999). Black mulberry fruits have a higher phenolic content (342.43 mg GAE/100 g FW) than raspberry fruits (242.96 mg GAE/100gFW). Previously, a wide variation was observed in the total phenolic content in fruits of raspberry of 1280-2116 mg Gallic acid equivalents per g DW basis (Pantelidis *et al.*, 2007) and 178.6- 186.1 mg Gallic acid equivalents per 100 g FW basis (Bobinaite *et al.*, 2012). Phenolic content data from mulberry fruits obtained are comparable to previous finding which reported values between 173-342 mg GAE /100gFW for some cultivated mulberry and between 168- 388 mg GAE /100g FW for wild Turkian mulberries (Radojkovic *et al.*, 2010; Arfan *et al.*, 2012). Our total phenolic results were higher than those reported elsewhere. The phenolic content and composition of fruits depend on environmental factors as well as post-harvest processing conditions (Benvenuti *et al.*, 2004; Kadir *et al.*, 2009).

Flavonoids are considered as phenol compounds with highest antioxidant activity due to their chemical structure (Kahkonen *et al.*, 1999). The total flavonoid content in investigated samples was in the range of 165-178.62 mg Q/100gFW (Table 1).

Table 1. Total phenolic (TP), total antioxidant activity (TAA), ascorbic acid (AA), total anthocyanin (TA) and total flavonoids content (TF) of mulberry and raspberry fruits.

species	TAA (%)	TA (mg/100gFW)	TP (mgGAE/100gFW)	TF (mg Q/100gFW)	TF/TP	AA (mg/100)
M. nigra	88.43	75.7 ± 2.95	345.77 ± 2.60	165.33 ± 1.52	0.47	29.58 ± 0.22
R. caesius	84.48	50.14 ± 1.12	242. 97 ± 4.78	178.67 ± 4.04	0.73	24.64 ± 0.22

The method of scavenging of stable DPPH˙ radicals is a method widely used to evaluate antioxidant activity in relatively short time compared to other methods. The effect of antioxidants on DPPH˙ radical scavenging was attributed to their hydrogen donating ability (Bauman *et al.*, 1979). DPPH˙ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Singelton and Rossi., 1965). Decrease in absorbance of DPPH˙ radical is caused by a reaction between antioxidant molecules and the radical, which results in the scavenging of radical by hydrogen donation, what is visually noticeable as a discoloration from purple to yellow.

The antioxidant activity results using DPPH method in raspberry and mulberry species are shown in Table 1. A statistical significant difference ($P< 0.05$) was found among species. Mulberry and raspberry species showed high antioxidant activity. The highest antioxidant activity was observed in *M. nigra* at 84.48.

Mulberry showed a significantly higher total anthocyanin content (75.7 mg /100gFW) than the raspberry (Table 1). The total anthocyanin content of red grape species were 6.9-15.1 mg per 100 g FW (Cantos *et al*., 2002), in mulberry species were 68.1-72.5 mg per 100 g (Ersisly *et al*., 2009) in red currants were 7.5-7.8 mg per 100 g (Pantelidis *et al*., 2007) in red raspberry 16.23-56.11 mg per100 g FW (Bobinaite *et al*., 2012). Our results were comparable with these results and it can be concluded that mulberry species were found to be good sources of anthocyanins among fruit species. The anthocyanin content and composition of fruits depended on environmental factors as well as post-harvest processing conditions (Benvenuti *et al*., 2004; Kadir *et al*., 2009). The fruits of mulberry and raspberry revealed the presences of considerable amounts of flavonoids. Thus, results of the present study supported the antioxidant and nutraceutical potential of this plant species. Ratio of total flavonoid / phenolics in the mulberry and raspberry fruits are presented in Table 1. The highest ratio total flavonoids / phenolics were observed in *R. caesius* at 0.73.

The average ascorbic acid content in mulberry and raspberry was 29.58 and 24.64 mg per 100 mL, respectively (Table 1). Lale and Ozcagiran (1996) reported that vitamin C content in black and purple mulberries was 26.6 and 11.9 mg/100 mL, which is in accordance with our results. Ascorbic acid content of raspberry was previously reported as being between 18.5 and 30 mg per 100 g (pantelidis *et al*., 2007; Liagat Ali *et al*., 2011; Rumune *et al*., 2011).

The correlation between measured parameters in raspberry and mulberry species is shown in Table 2. In the literature, the correlation between antioxidant activity and phenolic content has been reported in fruits of raspberry (Erika *et al*., 2011; Liagat Ali *et al*., 2011), strawberry species (sara *et al*., 2008) and red grape cultivars (Hulya *et al*., 2007).

Table 2. Pearson's correlation coefficients for quantitative determinations in raspberry and mulberry species.

Variable	TAA	TA	TP	TF	AA
TAA	1	0.96**	0.98**	0.96**	0.99**
TA	0.96**	1	0.99**	0.91*	0.98**
TP	0.98**	0.99**	1	0.92**	0.99**
TF	0.96**	0.91**	0.92**	1	0.95**
AA	0.99**	0.98**	0.99**	0.95**	1

[a]95% confidence interval, *significant at p< 0.05, **, significant at P<0.001.

As a conclusion, this investigation clearly shows the potential value of raspberry and mulberry germplasms. Berry fruits are a significant source of phenolic compounds, anthocyanins, total flavonoids and ascorbic acids. Antioxidant activity was high in fruits. Therefore mulberry and rasperry could be considered a good source of natural antioxidants. They can potentially be used in food and nutraceutical supplement formulations as well. Moreover, since commercial raspberry cultivars do not exist, these results could be important to use these species as breeding materials in future traditional breeding or advanced biotechnology studies. In addition, a wide range of agronomic characteristics, such as high yield and pest and disease resistance of these selected species could be incorporated into an improved raspberry cultivar.

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