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Antioxidant capacity and phytochemical properties of raspberry species in Iran

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Abstract

Raspberry (*Rubus* sp) is a naturally growing in North of Iran. Antioxidant capacity, total anthocyanins, total phenols, ascorbic acid and total flavonoids of a number of selected raspberry species were investigated. The total phenolic contents of raspberry species were in the range of 414-683.25 mg Gallic acid per 100 g Fruit Weight. *R. hyrcanus* had the highest total antioxidant capacity (67.75) and total flavonoid (295.5 mg Quercetin per 100 g FW). The highest total anthocyanin was observed in *R. hyrcanus* (45.36 mg cyaniding-3-glucoside equivalents/100g FW). The range of ascorbic acid content of species was 15.63 -22.44 mg per 100 g FW. There are linear relationships between the antioxidant capacities with total phenols, total flavonoid and ascorbic acid. The present study demonstrates the potential of certain raspberry species, notably *R. hyrcanus*, for improvement of nutritional value through *germplasm enhancement programs*.

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Introduction

Raspberries are among the most popular berries in the world, which are consumed as fresh fruits and processed to jams, confitures and other products or as ingredients in various foods. The raspberry (*Rubus sp*) is the most commercially important species in Iran.

Numerous studies demonstrated that various phytochemical constituents of raspberry fruits exhibit a wide range of biological effects, including antioxidant, anti-carcinogenic, vasodilator and antimicrobial properties (Mullen *et al*., 2002; Paredes-lopez *et al*., 2010). The most significant health benefits of raspberry fruits are attributed to the phenolic compounds, such as flavonoids, phenolic acids and tannins (Paredes-lopez *et al*., 2010). For instance, Ovaskainen *et al*. (2008) estimated a dietary intake and major food sources of polyphenols in Finnish adults and concluded that among 143 food items, berries were superior in terms of polyphenol concentrations. Due to a high content and wide diversity of phenolic compounds and their health-promoting properties, berries are often regarded as natural functional products. Raspberries, as possessing high antioxidant potential fruits, are a valuable source of potentially healthy compounds (Beekwilder *et al*., 2005). The antioxidant properties of raspberries are associated with a high content of anthocyanins, ellagic acid derivatives and ascorbic acid (Mullen *et al*., 2003).

Increasing recent interest in nutraceuticals and functional foods has led plant breeders to initiate selection of crops with higher than normal phenolic antioxidant contents, such as raspberries (shiow wang *et al*., 2009), plums and peaches (Cavallos-Casals *et al*., 2006) and strawberries and apples (Scalzo *et al*., 2005). All these programs aim to set the base line for establishing breeding efforts, with the intention of adding value to fruits, with respect to the level and diversity of health benefits that such crops could impart. In recent years increasing attention has been paid by consumers to the lesser known fruits such as raspberry, cornelian cherry, honeysuckle, hardy kiwifruit, lingonberry, elderberry, bilberry, strawberry, etc., which have unusual flavors and qualities, and many of which are rich with antioxidants and anthocyanins (Erisli *et al*., 2007). Therefore, detailed information about the health-promoting components of more raspberry species could lead to a better understanding and increased consumption of this fruit, including its use in functional foods and ingredients in pharmaceuticals, nutraceuticals, and medicine.

Raspberry (*Rubus sp*) fruit 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 fruit as the case is for other fruit species. The objective of this study was to determine antioxidant capacity, total anthocyanins, total phenolic, ascorbic acid, and total flavonoids of a number of selected raspberry fruits in Iran.

Materials and methods

Collection and preparation of raspberry fruits samples

Iranian raspberries that were evaluated in this study (*R. hircanus, R. astarae, R. persicus*) were collected from the north (Heiran, Caspian, Gilan province) regions of Iran.

Approximately 500 g of ripe raspberry fruits were harvested manually in July 2012. The fruits were sorted according to uniformity of shape and color and then immediately transported to lab and free zed with liquid nitrogen and kept at -80 ºC, until needed for analysis.

Extraction and measurement of total ascorbic acid

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 4ºC 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.

Extraction and measurement of total anthocyanins

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).

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.

Extraction and measurement of 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.

Extraction and measurement of total flavonoid

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.

Determination of the antioxidant capacity 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

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

Determination of the antioxidant capacity by FRAP assay

The FRAP assay (Benzie and Strain 1999) was conducted using three aqueous stock solutions containing 0.1 moll^{-1}acetate buffer (pH 3.6), 10 mmoll^{-1} TPTZ [2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine] acidified with concentrated hydrochloric acid, and 20 mmoll^{-1}ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1, v/v/v) to form the FRAP reagent just prior to analysis. For each assay laboratory duplicate, 2.97 ml of FRAP reagent and 30µl of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm on a spectrophotometer. The result was compared with the standard curve obtained by using different concentrations of $FeSO_4$. $7H_2O$.

Statistical analysis

Statistical analyses were performed using the SPSS for Windows version 16.0 (SPSS Inc.,USA). Differences among the means were compared between species using one-way analysis of variance. Multiple-comparison was done using either Tukeys or Dunnett·s T3 test. Differences at P< 0.05 were considered to be significant. The Pearson correlation test was conducted to determine the correlations among the means.

Results and discussion

The differences in total phenolic contents (TPC), total anthocyanins, antioxidant activity, total flavonoid and ascorbic acid among raspberry species were statically significant (P <0.05). The total phenolic content of raspberry species was in the range of 414-683.25 mg Gallic acid per 100 g FW basis. *R. hyrcanus* species has a greater TPC than other species (Fig. 1). 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 278.6- 496.1 mg Gallic acid equivalents per 100 g FW basis (Rumune *et al.*, 2011) . 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).

Table 1. Ascorbic acid (AA), total anthocyanin (TA) and ratio of total flavonoid / phenolics (TF/ TP) of raspberry fruits.

Species	TA (mg/100gFW)	AA (mg/100g)	TF/TP
R. hyrcanus	45.36 ^{b}	22.44 ^{a}	2.30
R. persicus	41.81 ^{c}	17.06 ^{b}	2.36
R. astarae	44.34 ^{a}	15.63 ^{c}	1.95

Results are expressed as a mean of three replicate measurements. The values with the different letter differ significantly (P<0.05).

The radical scavenging activity of fruits was determined from the reduction in the optical absorbance at 517 nm due to scavenging of stable DPPH free radical. The antioxidant activity results using DPPH method in raspberry species are shown in Figures 3. A statistical significant difference (P< 0.05) was found among species. Raspberry species showed high antioxidant activity. The highest antioxidant activity was observed in *R. hyrcanus* at 67.75 %, followed by *R. astarae* (51.5 %) and *R. persicus* (42.5 %). In order to quantify the antioxidant capacity, the IC_{50} which is the concentration of sample required to decrease the absorbance at 517 nm by 50%, was further calculate and is shown in Figures 4. *R. hyrcanus* species had the lowest IC_{50} values (86.23 µg ml^{-1}). The FRAP assay showed greater variability between species (Fig. 5). The raspberry species had FRAP values in the range 26.1-40.53 µmol Fe^{+2}/ g FW. R. hyrcanus species was significantly more active than other species (P<0.05). The content of total anthocyanins of raspberry species ranged from 41.81 to 45.36 mg, expressed as cyanidin3-glucoside equivalents per 100 g FW basis (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 gooseberry species were 1.4-7.5 mg per 100 g 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 (Rumune *et al.*, 2011). Our results were comparable with these results and it can be concluded that raspberry 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).

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

Variables	TAA	TA	TP	TF	AA
TAA	1	0.834**	0.991**	0.874**	0.846**
TA		1	0.863**	0.906**	0.52ns
TP			1	0.919**	0.798**
TF				1	0496ns
AA					1

TAA: total antioxidant capacity by DPPH radical scavenging method, TA: total anthocyanins, TP: total phenolics, TF: total flavonoid, AA: ascorbic acid, ns: no significant;*P<0.05%,**P<0.01%.

The results for total flavonoid content are shown in Figures 2. The total flavonoid contents of raspberry species were in the range of 175-296.5 mg Quercetin per 100 g FW basis. Results indicated that the difference in flavonoid content among raspberry species were statistically significant (P<0.05). The fruits of 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. The differences in the composition of the fruits could be due to the growing conditions, such as soil, geographical and environmental conditions during the fruit development, degree of maturity at harvest and genetic differences (Agata *et al.*, 2009). Ratio of total flavonoid / phenolics in the raspberry fruits are presented in Table 1. The highest ratio total flavonoids / phenolics were observed in *R. persicus* at 2.36.

A wide variation was found among raspberry species in terms of ascorbic acid content, ranging from 15.63 mg to 22.44 mg per 100 g. The R. hyrcanus had the highest ascorbic acid content in its fruits (22.44 mg per 100 g). 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 species is shown in Table 2. No statistically significant correlation was observed between total anthocyanins and ascorbic acid. 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).

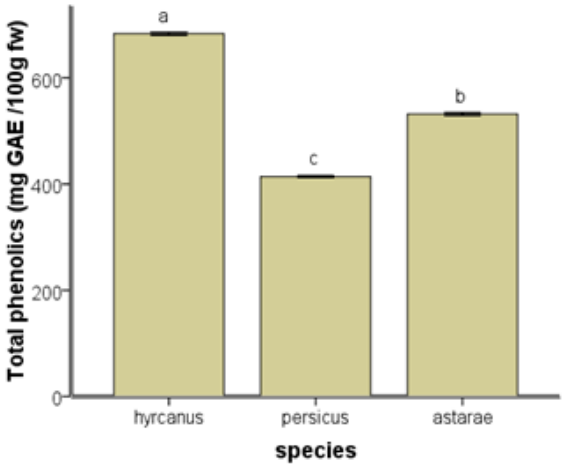

Fig. 1. Total phenolic content (TPC) in raspberry species. Results are expressed as mg GAE / 100 g FW. The means marked by different letters are significant differences according to the Tukey test (P<0.05).

As a conclusion, this investigation clearly shows the potential value of raspberry germplasm. Raspberry fruits are a significant source of phenolic compounds,

anthocyanins, total flavonoids and ascorbic acids. Antioxidant activity was high in fruits and varied greatly among the species. Therefore raspberry 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.

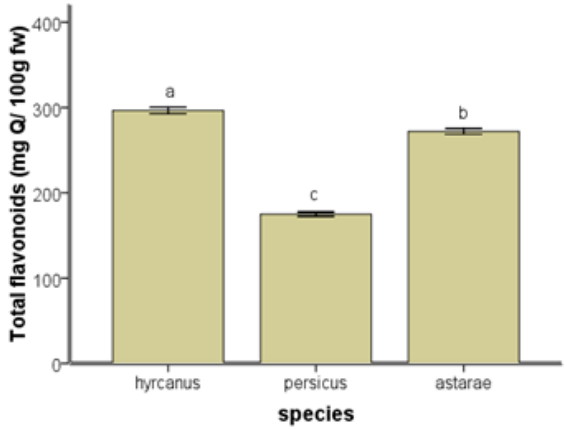

Fig. 2. Total flavonoid content (TFC) in raspberry species. Results are expressed as mg Quercetinper 100g FW. The means marked by different letters are significant differences according to the Tukey test (P<0.05).

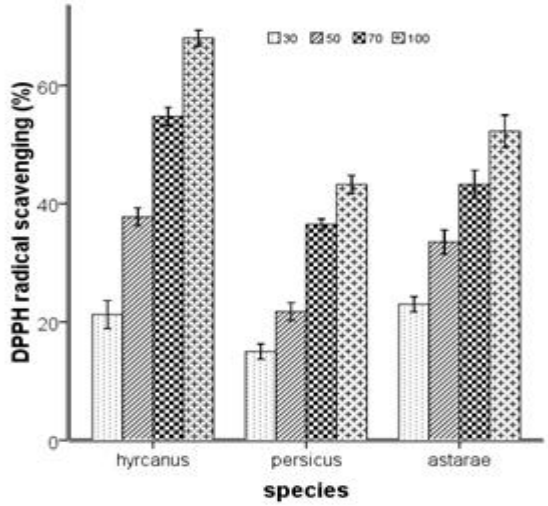

Fig. 3. DPPH free radical scavenging capacity in raspberry species. Results are expressed as mg Quercetinper 100 gFW. Various concentrations of extracts (30, 50, 70 and 100 µg ml-1) were assayed in 10 min.

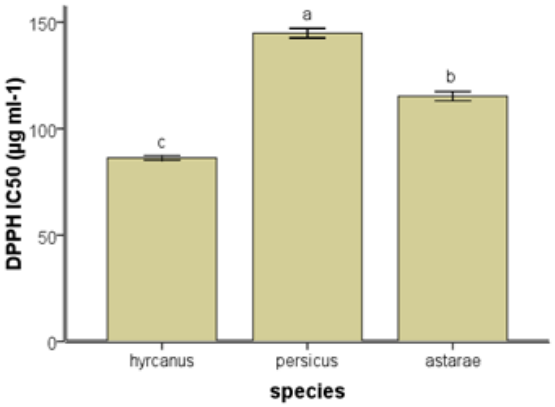

Fig. 4. Amounts of raspberry species extracts needed to scavenge DPPH free radical by 50%. Values are expressed as µg ml-1(ppm). The means marked by different letters are significant differences according to the Tukey test (P<0.05).

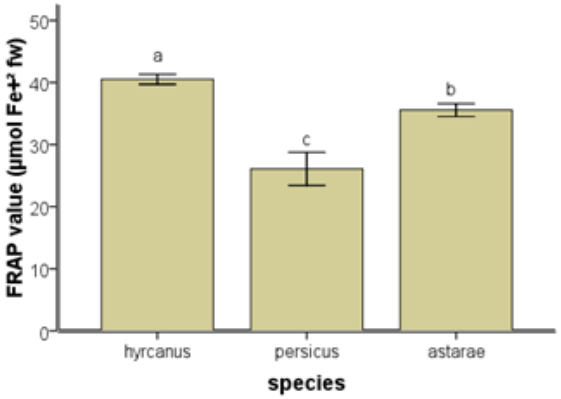

Fig. 5. Ferric-reducing antioxidant power in raspberry species. Results are expressed as micro molar Quercetin equivalents per 100g FW. The means marked by different letters are significant differences according to the Tukey test (P<0.05).

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