Pomological Features, Nutritional Quality, Polyphenol Content Analysis, and Antioxidant Properties of Domesticated and 3 Wild Ecotype Forms of Raspberries (*Rubus idaeus* L.)

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Abstract: The raspberry (*Rubus idaeus* L.) is an economically important berry crop that contains many phenolic compounds with potential health benefits. In this study, important pomological features, including nutrient content and antioxidant properties, of a domesticated and 3 wild (Yayla, Yavuzlar, and Yedigöl) raspberry fruits were evaluated. Also, the amount of total phenolics and flavonoids in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits were calculated as gallic acid equivalents (GAEs) and quercetin equivalents (QE). The highest phenolic compounds were found in wild Yayla ecotype (26.66 ± 3.26 GAE/mg extract). Whilst, the highest flavonoids were determined in wild Yedigöl ecotype (6.09 ± 1.21 QA/mg extract). The antioxidant activity of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits were investigated as trolox equivalents using different *in vitro* assays including DPPH$^{\bullet}$, ABTS$^{\bullet+}$, DMPD$^{\bullet+}$, and $O_2^{\bullet-}$ radical scavenging activities, H_2O_2 scavenging activity, ferric (Fe^{3+}) and cupric ions (Cu^{2+}) reducing abilities, ferrous ions (Fe^{2+}) chelating activity. In addition, quantitative amounts of caffeic acid, ferulic acid, syringic acid, ellagic acid, quercetin, α-tocopherol, pyrogallol, p-hydroxybenzoic acid, vanillin, p-coumaric acid, gallic acid, and ascorbic acid in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits were detected by high-performance liquid chromatography and tandem mass spectrometry (LC-MS-MS). The results clearly show that p-coumaric acid is the main phenolic acid responsible for the antioxidant and radical scavenging activity of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits.

Keywords: antioxidant activity, pomological features, radical scavenging, raspberry, *Rubus idaeus*

Introduction

Fruits and vegetables are a primary food source providing essential nutrients for sustaining life. They contain a variety of phytochemicals, such as phenolic acids and flavonoids, which have been associated with many health benefits. Hence, regular consumption of fruits and vegetables has been associated with reduced risks of chronic diseases, such as cancers and cardiovascular disease (Isabelle and others 2010). Antioxidants are reducing agents and limit oxidative damage to biological structures by scavenging free radicals. When they are added to lipids and lipid-containing foods, their shelf-life can be increased by retarding the process of lipid peroxidation (Eberhardt and others 2000).

Raspberries (*Rubus ideaus* L.) are a member of the Rosaceae family, grown as a perennial crop. They are grown in many parts of the world and constitute an important high-value horticultural industry in many European countries providing employment in agriculture and indirectly in food processing. Turkey has a very rich and wide range of native fruit species, mainly in different climatic regions in northeast Turkey and localities of the Çoruh Valley (Davis 1982).

Red raspberry (*R. idaeus* L.) contains numerous phenolic compounds with potential health benefits (De Ancos and others 2000). They are soft, juicy with a distinct aroma and are a good source of natural antioxidants. In addition to vitamins and minerals, raspberries are also rich in anthocyanin, phenolic acids, and other flavonoid (Wang and Lin 2000, Mullen and others 2002, Beekwilder and others 2005).

Currently, there is an increasing interest in raspberries as a major source of antioxidants, such as anthocyanin, catechin, flavonol, flavone, and ascorbic acid, as they may offer protection against a wide variety of human diseases (Stewart and others 2007). However, the amount and content of the phenolic compounds varies significantly between cultivars (Anttonen and Karjalainen 2005). Although the health-promoting components of mainly cultivated raspberries were reported (Kafkas and others 2008); to our best knowledge, there are only a few reports dealing with the antioxidant potency and bioactivity of compounds in wild raspberries. Detailed information about the health-promoting components of wild raspberries is needed to give a better insight into their use as functional foods and as ingredients in pharmaceuticals, nutraceuticals, and medicines.

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In this study, the ferric ions (Fe^{3+}) reducing antioxidant power assay, cupric ions (Cu^{2+}) reducing antioxidant power assay (Cuprac method), DPPH$^{\bullet}$ radical scavenging, ABTS$^{\bullet+}$ radical scavenging, DMPD$^{\bullet+}$ radical scavenging, superoxide anion radical scavenging, hydrogen peroxide (H_2O_2) scavenging, and ferrous ions (Fe^{2+}) chelating activities of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits were investigated. These multiple methods are recommended to measure antioxidant properties of food materials to reflect their potential protective effects. Furthermore, some pomological features of the polyphenol contents of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits were clarified.

Materials and Methods

Plant material

A domesticated raspberry cultivar (*R. idaeus* L.) (Willamette) and 3 advanced selections (Yedigöl, Yayla, Yavuzlar) from the southwestern region of the Kackar Mountains, northeast Turkey were evaluated. Wild forms of ecotype raspberry were collected from in Ispir, northeast Turkey, and a native raspberry population with red color was collected from mount Kaçkar (altitude: 2,200 and 1,650 m, wild Yedigöl ecotype and Yavuzlar valley in southwest of the Kaçkar Mountain, northeast Turkey). In the Ispir region, raspberries grow over 1,200 m, having rich organic substance were collected.

Field experiments

Field studies were carried out on an experimental farm at Atatürk Univ., Ispir Technical Vocational School in northeast Anatolia (29 °55′N and 41 °16′E with an altitude of 1,950 m). In order to investigate the crop quality and phenological content of the raspberry cultivars, Willamette and 3 wild forms of raspberries where analyzed. Soil samples were taken from 20- to 40-cm depth and analyzed before plantation. The experimental soil was sandy-loamy with 3.1% organic matter and 0.98% lime content (pH: 6.8). Each planting was arranged in a randomized complete-block design, in which selections and cultivars were represented by 3 replications with 1-m space between plants and 2 m between rows in mid-August 2001 and 2004. The 2001 and 2004 plantings were evaluated for 3 y, starting in 2003 and 2006, respectively. In the 1st study (2001 to 2008), the aim was to examine the production and phenological criteria of raspberry species used for adaptation experiments in Ispir conditions in Erzurum as a part of adaptation and breeding projects carried out in northeast Turkey. Eighteen wild raspberry populations from the Çoruh region were evaluated. Except Willamette, other wild ecotype forms were grown widely and had valuable biotypes that were characterized by diversity. In the 2nd study (2004 to 2008) of the field experiment, the canes bore fruit and samples were collected.

The burst date of vegetative buds was between the 1st and end of April, while the appearance of flower clusters occurred between the end of May and the 1st of June. Duration of water applications to each plot was timed to ensure equal amounts of water were supplied to each. The 1st fruit maturation took place in the middle of June, and the 1st harvest was between the end of June and the 1st of July. The last harvesting date was in the middle of August, while the end of maturity was at the end of August.

Chemicals

The compounds used for antioxidant activity such as neocuproine (2,9-dimethyl-1,10-phenanthroline), N,N-dimethyl-p-phenylenediamine (DMPD), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), nitroblue tetrazolium (NBT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH$^{\bullet}$), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), riboflavin, and methionine were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and obtained from either Sigma-Aldrich or Merck (Darmstadt, Germany).

The following compounds were used as standards in LC-MS-MS analysis: caffeic acid (98%, Sigma-Aldrich), ferulic acid (98% Sigma-Aldrich), syringic acid (97%, Fluka Chemie GmbH, Steinheim, Switzerland), ellagic acid (95%, Fluka), quercetin (98%, Sigma-Aldrich), α-tocopherol (98%, Fluka), catechol (99% Sigma-Aldrich), pyragallol (98%, Sigma-Aldrich), p-hydroxybenzoic acid (99%, Merck), vanillin (99% Merck), p-coumaric acid (98%, Sigma-Aldrich), gallic acid (98%, Sigma-Aldrich), and ascorbic acid (99%, Sigma-Aldrich). Stock solutions were prepared as 5 mg/L in ethanol, except for catechol and ascorbic acid, which were prepared as 50 mg/L and 25 mg/L, respectively, in the same solvent. Curcumin (97%) and high-performance liquid chromatography (HPLC) grade methanol were purchased from Merck. Calibration solutions were prepared in ethanol-water (50:50, v/v) in a linear range. Dilutions were performed using automatic pipettes and glass volumetric flasks (A class). After preparation, standards were stored at –20 °C in glass containers. A curcumin stock standard was freshly prepared to contain 1,000-μg/L curcumin. A 100-μL portion of this was added to each sample for use as an internal standard (IS) in all LC-MS-MS experiments.

Pomological features and nutritional parameters

Red raspberry cultivars "Willamette" and wild selections (Yedigöl, Yayla, Yavuzlar) from the Çoruh region were evaluated for pomological traits such as cane length (cm) and diameter (mm), fruit weight (FW), total soluble solid content (%), total soluble (%) and reducing sugars (%), pH, titratable acidity (%), vitamin C (mg/100 mg), and total antioxidant capacity. The average FW for the season was calculated by the weight of a randomly selected 50-fruit subsample from every one of the 3 plots for each raspberry on each harvest and the yield for each harvest. Soluble solids were measured by a digital reflactometer and total acidity for red raspberries was determined by titration. After harvest, the samples were immediately transferred to the Research Laboratory in the Science Faculty at Atatürk Univ. in Erzurum and kept frozen at –20 °C until used.

Berry weight was determined by a digital balance. Soluble solid content of berries expressed as percentage was determined by a digital refractometer (A.Krüss-optronic Model DR201–95, Hamburg, Germany) at 22 °C. Total acidity (%) as citric acid and pH was determined by the AOAC method (2005).

Preparation of aqueous extracts of raspberries

The extraction procedure used has been previously described in detail (Gülçin and others 2005a; 2008). Briefly, berries were extracted with water by 1st homogenizing, 130 g of each raspberry sample in a blender. The homogenate was mixed with 200 mL of distilled water by a magnetic stirrer for 15 min. Then the aqueous extract was filtered through cheesecloth and Whatman No. 1 paper. The filtrates were frozen at –84 °C in an ultralow temperature freezer (SANYO Electric Co., Ltd., JAPAN) and lyophilized in a freeze drier under 5-mmHg pressures at –50 °C (Labconco, Freezone, Kansas City, Mo., U.S.A.).

Determination of total phenolic content

The total phenol content of the lyophilized aqueous extracts of the domesticated and wild ecotypes of raspberry fruits were determined by using the method previously described by Gülçin and others (2004) and Singleton and Rossi (1965). The total phenolic content in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample. Standard calibration curves were determined from the analysis of solvent standards containing gallic acid at different concentrations ranging from 100- to 500-μg gallic acid (correlation coefficient [r^2]: 0.9711).

$$Absorbance = 0.0012 \times [Phenolics] + 0.0069, \quad r^2 : 0.9711.$$

The content of total phenolic in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was calculated by employing a standard curve as prepared above using gallic acid and expressed as micrograms of GAEs.

Determination of total flavonoid contents

The total phenolic content in the lyophilized aqueous extracts of the raspberries was determined using the aluminum chloride colorimetric method described by Köksal and Gülçin (2008). The flavonoid content was calculated using a standard calibration curve prepared from the analysis of quercetin solution and expressed as micrograms of quercetin equivalent (QE) per gram of sample.

$$Absorbance = 0.0141 \times [Flavonoids] + 0.0042.$$

The content of total flavonoid in the lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was calculated from the above standard curve prepared using quercetin and expressed as micrograms of QEs. Standard calibration was made from 10- to 50-μg quercetin (r^2: 0.9939).

Preparation of test solution for LC-MS-MS

One hundred milligrams of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was dissolved in 5 mL of ethanol-water (50:50 v/v) in a volumetric flask, from which 1 mL was transferred into another 5 mL of volumetric flask. Then, 100 μL of curcumin was added and diluted to the volume with ethanol-water (50:50 v/v). From the final solution, 1.5 mL of aliquot was transferred into a capped autosampler vial and 10 μL of sample was injected onto LC column. Samples in the auto-sampler were kept at 15 °C during the experiment.

Instruments and chromatographic conditions

Experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometer equipped with a Macherey-Nagel Nucleoder C18 Gravity column (Machery-Nagel, Easton, Pennsylvania, Pa., U.S.A.; 125 × 2 mm i.d., 5 μm particle size). The mobile phase was composed of methanol (A, 0.5% formic acid) in water (B, 0.5% formic acid), the gradient program was 0 to 1.00 min 50% A and 50% B, 1.01 to 30.00 min 100% A, and finally 30.01 to 35.00 50% A and 50% B. The flow rate of the mobile phase was 0.3 mL/min, and the column temperature was set to 30 °C. The injection volume was 10 μL.

Optimization of HPLC methods and LC-MS-MS procedure

The best mobile phase composition was determined to be a gradient of acidified methanol and water. This mobile phase was shown to be satisfactory for the ionization and separation of the compounds. As the analysts of interest were small and/or polar in nature, electrospray ionization (ESI) was selected over atmospheric pressure chemical ionization (APCI) (Gören and others 2009). Quantification was performed via selected ion monitoring (SRM) on a triple quadrupole mass spectrometer. The optimum MS parameters were determined as 2.40 mTorr collision induced dissociation (CID) gas pressure, 5,000-V ESI needle voltage, 600-V ESI shield voltage, 300 °C drying gas temperature, 50 °C atmospheric pressure ionization (API) housing temperature, 55 psi Nebulizer gas pressure, and 40 psi drying gas pressure. Detailed information on experiment parameters is given in Table 2.

Validation of experiments and uncertainty evaluation

The detector linearity for each compound for the reported method was determined by analyzing standard solutions. The linearity range was determined as 0 to 1 mg/mL for compound 2, 3, 4, 5, 8, 9, 11, and 12, 0.05 mg/mL for compound 1, 0 to 2.5 mg/mL for compound 6, 1 to 25 mg/mL for compound 7, and finally, 0.1 to 10 mg/mL for compound 13. The r^2s were determined as $\geq$ 0.990. The linear regression equation for the reported compound was used for the calculation of concentration in the plant extract.

The recoveries of experiments were evaluated at three fortification levels (0.25, 0.5, and 1 mg/mL). Detailed information was reported in the literature (Gülçin and others 2010a). The precision of the reported method was evaluated by repeating the measurements 6 times at 3 different concentrations. Finally, a LOD and limits of quantification (LOQ) for the reported method for the above compounds were calculated to be 0.5 to 50 μg/L. The LOQs were determined to be 3 times bigger than LOQ.

The concentration of each analyte within the linear range and concentration of the reported method was obtained from the calibration curve. Finally, the calculated concentrations were converted to mg/kg of crude sample by the below equation.

$$\text{Amount(mg/kg)} = \left(\frac{C_a \times V_{Final}}{m \times V_{Final}}\right) \times 1000,$$

where C_a is the analyte concentration obtained by calibration curve (in μg/L), V_{final} is the final diluted volume before the analysis, m is amount of extract as gram, $V_{initial}$ is the initial sample volume. The EURACHEM/CITAC guide was used for evaluation of sources and quantification of uncertainty of LC-MS-MS method (EURACHEM/CITAC 2000). The maximum contribution comes from the calibration curve. Detailed procedures of uncertainty evaluation were reported previously in the literature (Gören and others 2007).

$$U_{rel}(Con) = \sqrt{u^2rel(C_a) + u^2rel(m_a) + u^2rel(m_{is}) + u^2rel(V_{final}) + u^2rel(V_{initial})},$$

where, C_a is the uncertainty from the calibration curve, V_{final} is the final volume of the sample, V_{init} is the initial volumes of IS, sample weighing of m_a is the weighing of analyte and m_{is} is the weighing of IS (Gülçin and others 2010a). The relative uncertainties have been found to be in the range of 1.6% and 7.8% at 95% confidence level (k: 2) (Table 2).

Fe^{3+} reducing power assay

Reducing power of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was measured by the direct reduction of $Fe^{3+}(CN^{-})_6$ to $Fe^{2+}(CN^{-})_6$ and was determined by measuring absorbance resulting from the formation of the Perl's Prussian Blue complex following the addition of excess Fe^{3+} (Gülçin 2006a). Different concentrations of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits (20 μg/mL) in 0.75 mL of distilled water were mixed with 1.25 mL of 0.2 M, pH 6.6 sodium phosphate buffer, and 1.25 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50 °C for 20 min. After 20 min of incubation, the reaction mixture was acidified with 1.25 mL of trichloroacetic acid (10%). Finally, 0.5 mL of $FeCl_3$ (0.1%) was added to this solution, and the absorbance was measured at 700 nm in a spectrophotometer (Büyükokuroğlu and others 2001).

Cu^{2+} reducing-Cuprac assay

In order to determine the reducing ability of Cu^{2+} toward lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits, the method by Apak and others (2006) was applied. For this reason, 0.25-mL $CuCl_2$ solution (0.01 M), 0.25-mL ethanolic neocuproine solution (7.5×10^{-3} M), and 0.25-mL CH_3COONH_4 buffer solution (1 m) were added to a test tube, followed by mixing with the same concentration of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits (20 μg/mL). The total volume was then adjusted to 2 mL with distilled water and mixed thoroughly. The tubes were stoppered and kept at room temperature for 30 min. Absorbance was measured at 450 nm against a reagent blank after the 30 min resting period. Increased absorbance of the reaction mixture indicates increased reduction capability. The Cu^{2+} reducing power of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was determined as trolox equivalents (Gülçin and others 2010c).

Chelating activity on Fe^{2+}

Fe^{2+} chelating activity was measured by inhibiting the formation of Fe^{2+}-ferrozine complex after treatment of test material with Fe^{2+}, following the method of Dinis and others (1994). Briefly, 20-μg/mL concentrations of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits in 0.4-mL methanol were added to a solution of 0.6-mM $FeCl_2$ (0.1 mL). The reaction was initiated by the addition of 5-mM ferrozine (0.1 mL) dissolved in methanol. Then, the mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm (Gülçin and others 2006). The ability of each antioxidant to inhibit the formation of the ferrous iron-ferrozine complex, expressed as its Fe^{2+} chelating activity. The control contains only $FeCl_2$ and ferrozine (Gülçin 2007). The Fe^{2+} chelating effect of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was determined as trolox equivalent

$DPPH^{\bullet}$ scavenging activity

$DPPH^{\bullet}$ solution (0.1 mM) was prepared in ethanol, and 0.5 mL of this solution was added to 1.5 mL of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruit solutions in ethanol at different concentrations (20 μg/mL). These solutions were vortexed thoroughly and incubated in dark for 30 min. Then, the absorbance was measured at 517 nm against blank samples lacking scavenger (Gülçin and others 2005b).

$ABTS^{\bullet+}$ scavenging activity

The $ABTS^{\bullet+}$ was produced by reacting 2-mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$) stored in the dark at room temperature for 6 h (Gülçin 2010). The $ABTS^{\bullet+}$ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0.1-M sodium phosphate buffer (pH 7.4). Then, 1 mL of $ABTS^{\bullet+}$ solution was added to 3 mL of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits solution in ethanol at different concentrations (20 μg/mL). The absorbance was recorded 30 min after mixing, and $ABTS^{\bullet+}$ scavenging was calculated for each concentration relative to a blank, containing no scavenger. The extent of decolorization is calculated as percentage reduction of absorbance (Gülçin and Dastan 2007).

Superoxide radical scavenging activity

Superoxide radicals were generated in riboflavin, methionine, illuminate and assayed by the reduction of NBT to form blue formazan (Gülçin and others 2010b). All solutions were prepared in 0.05-M phosphate buffer (pH 7.8). The photo-induced reactions were performed using fluorescent lamps (20 W). The concentration of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits in the reaction mixture was 20 μg/mL. The total volume of the reaction mixture was 3 mL, and the concentrations of the riboflavin, methionine, and NBT were 1.33×10^{-5}, 4.46×10^{-5}, and 8.15×10^{-8} M, respectively. The reaction mixture was illuminated at 25 °C for 40 min. The photochemically-reduced riboflavin generated $O_2^{\bullet-}$ that reduced NBT to form blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance was measured at 560 nm. Lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was added to the reaction mixture, in which $O_2^{\bullet-}$ was scavenged, thereby inhibited the NBT reduction.

$DMPD^{\bullet+}$ scavenging activity

Finally, antiradical capacity was analyzed by $DMPD^{\bullet+}$ assay. DMPD radical scavenging ability of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was performed as described in a previous study (Gülçin 2008). DMPD (100 mM) was prepared by dissolving 209 mg of DMPD in 10 mL of deionized water, and 1 mL of this solution was added to 100 mL of 0.1 M acetate buffer (pH 5.25), and the colored radical cation ($DMPD^{\bullet+}$) was obtained by adding 0.2 mL of a solution of 0.05-M $FeCl_3$. The absorbance of this solution, which is freshly prepared daily, is constant up to 12 h at room temperature. Different concentrations of standard antioxidants or lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits (20 μg/mL) were added in test tubes, and the total volumes were adjusted to 0.5 mL with distilled water. Ten minutes later, the absorbance was measured at 505 nm. One milliliter of $DMPD^{\bullet+}$ solution was directly added to the reaction mixture, and its absorbance was measured at 505 nm. The buffer solution was used as a blank sample (Ak and Gülçin 2008).

Statistical analysis

The experiments were performed in triplicate. The data were recorded as mean $\pm$ standard deviation and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc.). One-way analysis of variance (ANOVA) was performed. Significant differences between means were determined by Duncan's Multiple Range tests, and $p < 0.05$ was regarded as significant, and $p < 0.01$ was very significant.

Results and Discussion

Studies of the biochemical composition of the red raspberry population of northwest Anatolia allowed selection of the best cultivars characterized by the highest content of vitamins and sugar. The highest sugar content was found in wild Yavuzlar ecotype. The values for total soluble solid, total, and reducing sugar were higher in the wild Yavuzlar ecotype than in other wild raspberries. The values for titratable acidity and pH were similar for each wild raspberry, and the amount of vitamin C was smaller in wild Yayla ecotype than others

To our knowledge, there is no study of this kind in the available literature concerning the Çoruh Valley wild-form raspberry plants. Raspberries have a vast range of distribution in different regions of Turkey including Çoruh Valley, Artvin, and different climatic regions in northeast Turkey. Wild populations of red raspberry can be found in many places, along the roads, on the borders of forests, and in forests clearings. Raspberries are known as a valuable berry plant and wild raspberry can serve as a potential source of new genetic material for cultivated raspberry breeding (Ryabova 2007). Additionally, selection of favorable agronomic traits requires data from different seasons and environmental locations before any breeder selection can proceed to finished cultivar (Woodhead and others 2008).

The raspberry populations growing wild are quite winter-hardy and possess other valuable qualities that could be transferred to the cultivated raspberry (Viskelis and others 2006; Ryabova 2007). Yedigöl valley on the southwestern side of the Kaçkar Mountain has been one of the valuable ecoregions of northeast Turkey where red raspberries are collected and used as new genetic material for cultivated raspberry breeding. This is why, in this region, you may find valuable biotypes that grow in the wild and show the best winter-hardiness as well. To identify the wild raspberry forms, we compared them to cultivars (Willamette). The Willamette cultivar with the highest FW, cane yield, soluble solid, and total acids content is most commonly grown in central Anatolia (Eyduran and others 2007).

Table 1 shows some pomological characteristics of the raspberry species planted in 2004 and harvested in 2006 to 2008. The berries of wild Yavuzlar ecotype distinguish themselves with the significantly biggest amount of soluble solid (11.66%), total soluble sugars (22.03%), and reducing sugars (10.62%) contents. Total phenolic content found to be higher in the wild Yayla ecotype (26.66 ± 3.26 GAE/mg lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits) than in other raspberries. On the other hand, a higher amount of total flavonoid and extraction yield was found for wild Yedigöl ecotype: 6.09 ± 1.21 QE/mg lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits and 8.57% ± 12.16%, respectively. The value for titratable acidity for Willamette is higher than that of others.

The content of phenolic compounds (mg/g) in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was determined in the raspberries fruits and expressed as milligram of GAEs (Table 1). The total phenolic compounds in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits were found to be in the following order: wild Yayla ecotype (26.66 mg GAE) > wild Yedigöl ecotype (17.51 mg GAE) ≥ Willamette (15.64 mg GAE) > wild Yavuzlar ecotype (5.83 mg GAE). Phenolic compounds are likely to contribute to the radical scavenging activity of these plant extracts. The effect of freezing and storage on the phenolics in red raspberry fruits was investigated by Mullen and others in GAEs (2002). The overall levels of phenolics in extracts of the fresh, frozen, shop, and home raspberry samples were found between 3,510 and 3,769 nmol/g, respectively, were slightly higher than in the fresh and frozen samples (3,383 and 3,321 nmol/g), respectively. In another study, Wang and Lin (2000) evaluated oxygen radical absorbance capacity and total phenolic content of red raspberry (*R. idaeus*), black raspberry (*R. occidentalis*) and strawberry (*Fragaria ananassa*) plants. Total phenolic content of berries varied from 9.1 to 33.8 mg/kg for fresh fruits (91.6 to 231.0 mg/kg of dry matter) as GAEs. A positive and highly significant relationship between total phenolic and antioxidant activity was found in this study. In addition, the phenolic profiles of 26 berry samples were analyzed with HPLC. Evaluation of antioxidant activity was performed by autoxidazing methyl linoleate in the dark. However, there was not found any correlation between the phenolic composition and the antioxidant activity (Kahkonen and others 2001). Also, the health benefits of raspberries was evaluated in another merit study (Liu and others 2002). In this study, total antioxidant and antiproliferative activities, total phenolics and flavonoids for 4 raspberry (Heritage, Kiwigold, Goldie, and Anne) varieties was determined. The Heritage raspberry variety had the highest total phenolic content (5,127 mg/kg of GAE) of the varieties measured followed by Kiwigold (4,511 mg/kg of GAE), Goldie (4,275 mg/kg of GAE), and Anne (3,592 mg/kg of GAE). Recently, Pantelidis and others (2007) determined phenolic contents of raspberries, blackberries, and gooseberries. Total phenolic contents of the raspberries varied from 657 to 2,494 mg/kg of GAE.

The phytochemicals in plant responsible for the antioxidant capacity can largely be attributed to the phenolics, anthocyanins, and other flavonoid compounds (Wang and Lin 2000). Flavonoids are the most common group of polyphenolic compounds in human diet and are found ubiquitously in plants. They can prevent coronary heart disease and have antioxidant properties. The content of

Table 1–Some pomological analysis and total phenolic, flavonoid, and yield determination of red ripe fruit harvested from 4 red raspberry cultivars at the middle of August 2008.

	Wild Yavuzlar ecotype	Wild Yayla ecotype	Wild Yedigöl ecotype	Willamette ecotype
pH	3.66 ± 0.01^{a}	3.70 ± 0.02^{a}	3.65 ± 0.09^{a}	3.28 ± 0.03^{b}
Soluble solids*	11.66 ± 0.24^{a}	10.05 ± 0.42^{b}	11.28 ± 0.34^{b}	9.68 ± 0.24^{b}
Titratable acidity*	1.11 ± 0.01^{b}	1.05 ± 0.04^{b}	1.09 ± 0.05^{b}	1.34 ± 0.02^{a}
Total soluble sugars*	22.03 ± 1.25^{a}	15.94 ± 0.50^{c}	19.58 ± 0.71^{b}	15.56 ± 0.52^{c}
Reducing sugars*	10.62 ± 0.03^{a}	7.40 ± 0.08^{d}	10.27 ± 0.30^{b}	7.71 ± 0.10^{c}
Extraction yield*	6.31 ± 1.82^{a}	7.12 ± 1.33^{a}	8.57 ± 12.16^{b}	6.15 ± 0.94^{a}
Total phenolic acids§	5.83 ± 1.12^{a}	26.66 ± 3.26^{b}	17.51 ± 2.86^{c}	15.84 ± 3.11^{c}
Total flavonoidsψ	1.77 ± 0.032^{a}	2.41 ± 0.61^{a}	6.09 ± 1.21^{b}	2.62 ± 0.33^{a}

Different letters in the same line indicate significant differences ($p < 0.05$).
*It was expressed as percentage (%).
§It was calculated as GAE.
ψIt was calculated as QE.

flavonoid compounds in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was determined as milligram of QE (Table 1). The total flavonoids in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits were found to be in the following order: wild Yedigöl ecotype (6.09 mg QE) > Willamette (2.62 mg QE) ≈ wild Yayla ecotype (2.41 mg QE) ≈ wild Yavuzlar ecotype (1.77 mg QE). Liu and others (2002) determined total flavonoids for 4 raspberry (Heritage, Kiwigold, Goldie, and Anne) varieties as catechin equivalents (CEs). Similar to the phenolic contents, the Heritage raspberry variety contained the highest total flavonoids (1034 mg/kg of CE) of the varieties tested, followed by Kiwigold (873 mg/kg of CE), Goldie (842 mg/kg of CE), and Anne (635 mg/kg of CE).

Phenolic acids are plant metabolites spread throughout the plant kingdom. The recent focus of interest on phenolic acids comes from their potential protective role, through ingestion of fruits and vegetables, against oxidative damage diseases such as coronary heart disease, stroke, and cancers (Elmastas and others 2006). The profile of phenolic acids in lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was analyzed by UPLC-MS-MS. Referring to Table 2, it is clearly shown that p-coumaric acid is the predominant phenolic compound identified in free form in all raspberries (*R. idaeus* L.) species. On the other hand, 0.311-mg/kg p-coumaric acid was detected in frozen red raspberries under HPLC conditions (Mullen and others 2002). Among the raspberry (*R. idaeus* L.) species, wild Yavuzlar ecotype had the highest amount of curcumin (14.6 mg/kg), ferulic acid (4.9 mg/kg), syringic acid (7.3 mg/kg), ellagic acid (10.9 mg/kg), catechol (12.1 mg/kg), pyrogallol (9.7 mg/kg), and gallic acid (14.6 mg/kg), whereas wild Yayla ecotype had the highest amount of quercetin (80.0 mg/kg). On the other hand, wild Yedigöl ecotype had the highest amount of α-tocopherol (15.06 mg/kg). Willamette had the highest quantity of caffeic acid (5.3 mg/kg), ascorbic acid (5.3 mg/kg), and p-coumaric acid (2,792.6 mg/kg). p-Hydroxybenzoic acid was found in none of the raspberry (*R. idaeus* L.) species. Ascorbic acid was not detected in wild Yayla and Yedigöl ecotypes. It was reported that raspberries contain 250 mg/kg of vitamin C (Liu and others 2002). Therefore, the total antioxidant activity of raspberries was mainly from the other phytochemicals in the fruit rather than from the vitamin C. The antioxidant activity may be explained by looking at the combination of different phytochemicals functioning additively or synergistically accounting for the total antioxidant activity of raspberry.

As can be seen in literature, different antioxidant methods were used for determination of antioxidant activities of raspberry species (Wang and Lin 2000; Kahkonen and others 2001; Liu and others 2002). The antioxidant activity of raspberry cultivars was measured by using the total oxyradical scavenging assay and were expressed as the median effective dose (Liu and others 2002). In another study, Wang and Lin (2000) evaluated oxygen radical absorbance capacity of red raspberry (*R. idaeus*), black raspberry (*R. occidentalis*), and strawberry (*F. ananassa*) plants. Recently, antioxidant capacity, phenol, anthocyanin, and ascorbic acid contents in raspberries, blackberries, and gooseberries from the Mediterranean area of northern Greece were determined by Fe^{3+}-TPTZ reducing antioxidant power and deoxyribose protection assays (Pantelidis and others 2007).

Different antioxidant compounds may act through different mechanisms; consequently, one method alone cannot be utilized to fully evaluate the antioxidant capacity of foods and does not reflect antioxidant capacity of pure compounds. For this reason, different antioxidant capacity tests were carried out using different approaches and mechanisms (Gülçin 2006b). So, we used different methods in our study.

Lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits had more effective reducing power determined by using the potassium ferricyanide $[Fe^{3+}(CN^{-})_6]$ reduction and Cu^{2+} reducing methods when compared to the standard methods. Such an assay may indicate just how easily a given antioxidant donates electrons to reactive free radicals species, thus, promoting the termination of free radical chain reactions. Electron donation is an important means by which antioxidants promote the formation of less reactive species and may be assessed using the reducing power assay. To measure the reductive ability of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits, Fe^{3+}-Fe^{2+} transformation was investigated in the presence of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits using Oyaizu's method (1986). As can be seen in Table 3, lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits demonstrated powerful Fe^{3+} reducing ability as a trolox equivalent. The reducing power of the raspberry (*R. idaeus* L.) species was as follows: wild Yayla ecotype (0.658-μg TE) > wild Yedigöl ecotype (0.597-μg TE) > wild Yavuzlar

Table 2–LC-MS-MS parameters of selected compounds and amount of antioxidants in lyophilized aqueous extract of raspberries fruits in mg/kg concentration.

					Amount of antioxidants in the plant extracts (mg/kg)$^{\Psi}$			
nr	Compounds	Parent ion	Daughter ion	Collision energy (V)	Wild Yavuzlar ecotype	Wild Yayla ecotype	Wild Yedigöl ecotype	Willamette ecotype
S	Curcumin*	367	216.4	10	-	-	-	-
1	Caffeic acid	179	134.0	11	2.41 ± 0.19	-	-	5.31 ± 0.41
2	Ferulic acid	193	177.5	10	4.90 ± 0.18	-	-	-
3	Syringic acid	197	181.6	10	7.32 ± 0.23	0.10 ± 0.01	1.4 ± 0.04	2.11 ± 0.07
4	Ellagic acid	301	150.0	10	10.92 ± 0.28	1.01 ± 0.03	8.91 ± 0.23	5.32 ± 0.13
5	Quercetin	301	178.6	10	-	80.0 ± 1.31	-	-
6	α-Tocopherol	429	162.6	20	-	3.1 ± 0.11	15.61 ± 0.54	3.20 ± 0.11
7	Catechol	109	64.8	35	12.10 ± 0.29	-	8.91 ± 0.21	2.12 ± 0.05
8	Pyrogallol	125	78.7	20	9.70 ± 0.2	2.02 ± 0.04	8.90 ± 0.18	3.20 ± 0.07
9	p-Hydroxybenzoic acid	137	92.7	10	-	-	-	-
10	Vanillin	181	135.5	10	3.60 ± 0.07	3.0 ± 0.06	4.41 ± 0.09	3.22 ± 0.06
11	p-Coumaric acid	163	118.7	10	400.81 ± 15.95	67.03 ± 2.67	1145.6 ± 45.59	2792.60 ± 111.15
12	Gallic acid	169	124.6	10	14.6 ± 0.05	3.0 ± 0.06	6.7 ± 0.13	5.32 ± 0.10
13	Ascorbic acid	175	114.0	12	2.4 ± 0.1	-	-	5.34 ± 0.12

*Used as internal standard.
$^{\Psi}$LC-MS/MS analyses were replicated 6 times; (-) is below the limit of quantification.

ecotype (0.507-μg TE) > Willamette (0.442-μg TE). These results demonstrated that lyophilized aqueous extracts of domesticated and wild species of raspberry fruits had marked Fe^{3+} reducing ability and electron donor properties for neutralizing free radicals by forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging.

Cu^{2+} reducing ability of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was determined as trolox equivalent and is shown in Table 3. Cu^{2+} reducing power of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits and standard compounds at the same concentration (30 μg/mL) were as follows: wild Yayla ecotype (0.272-μg TE) > wild Yedigöl ecotype (0.221-μg TE) > wild Yavuzlar ecotype (0.195-μg TE) > Willamette (0.144-μg TE)

Metal-binding capacity was investigated by assessing the ability of the antioxidants to compete with the indicator ferrozine to complex with Fe^{2+} in solution. Lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits had a strong chelating effect on Fe^{2+}. The difference between different concentrations of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits (10 to 20 μg/mL) and the control values was statistically significant ($p < 0.01$). In addition, lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits exhibited effective chelation of Fe^{2+}. As seen in Table 3, the Fe^{2+} chelating effect of the domesticated and 3 wild (Yayla, Yavuzlar and Yedigöl) ecotypes of raspberry fruits was compared to that of BHA, BHT, α-tocopherol and trolox. The Fe^{2+} chelating capacity of the same concentration of wild Yayla ecotype, wild Yavuzlar ecotype, wild Yedigöl ecotype and Willamette ecotypes were found to be 0.934, 1.008, 0.937 and 0.981 trolox equivalents, respectively. These results show that the ecotypes have similar Fe^{2+} chelating effects.

DPPH radical scavenging effect of the domesticated and 3 wild (Yayla, Yavuzlar, and Yedigöl) ecotypes of raspberry fruits were found as 0.899, 0.903, 0.617, and 0.628 trolox equivalents, and decreased in the following order: wild Yavuzlar ecotype > wild Yayla ecotype > Willamette ≈ wild Yedigöl ecotype. A higher trolox equivalent value indicates a higher DPPH free radical scavenging activity.

All the tested samples exhibited effective radical cation scavenging activity. As seen in Table 3, lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits is an effective $ABTS^{\bullet+}$ radical scavenger. There is a significant decrease ($p < 0.01$) in the concentration of $ABTS^{\bullet+}$ due to the scavenging capacity at all lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits concentrations. On the other hand, the domesticated and 3 wild (Yayla, Yavuzlar, and Yedigöl) ecotypes of raspberry fruits exhibited 0.934, 1.007, 0.936, and 0.981 trolox equivalents, respectively. The scavenging effect of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits and standards on $ABTS^{\bullet+}$ decreased in the following order: wild Yavuzlar ecotype ≈ Willamette ≈ wild Yedigöl ecotype ≈ wild Yayla ecotype at the same concentration (30 μg/mL).

The inhibition of superoxide anion radical generation by 30-μg/mL concentration of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was found as trolox equivalent. At the same concentration, the domesticated and 3 wild (Yayla, Yavuzlar, and Yedigöl) ecotypes of raspberry fruits demonstrated 3.884, 3.296, 1.476, and 3.041 trolox equivalents as superoxide anion radical scavenging activity, respectively. Based on these results, the wild Yayla ecotype had higher superoxide anion radical scavenging activity than all other ecotypes.

As shown in Table 3, the domesticated and 3 wild (Yayla, Yavuzlar, and Yedigöl) ecotypes of raspberry fruits are effective $DMPD^{\bullet+}$ radical scavengers. These ecotypes demonstrated 1.928, 1.849, 2.065, and 1.634 trolox equivalents, respectively. The results showed that wild Yedigöl ecotype is the most effective $DMPD^{\bullet+}$ radical scavenger.

Selected native red raspberry fruits investigated in this study were shown to be a novel rich source of antioxidant compounds. This study demonstrated that Willamette berries and wild red raspberries have high potential value for fruit growers as well as the food and nutraceuticals manufacturers because of their high polyphenolic contents. The results clearly show that the antioxidant activity in raspberry is different among cultivars, selections, and families and could be improved by a breeding program. The present study indicates that the wild raspberry fruits are an extremely rich source of ascorbic acid, soluble sugar, phenols, and antioxidants, demonstrating its potential use as a food additive. Our study provides valuable information on the antioxidant capacity of wild red raspberry species grown in Çoruh Valley and highlights the crucial influence of cultivar on elemental content and antioxidant power of berry fruits. Furthermore, it also revealed the importance of the ability to select berry cultivars for specific nutritional purposes or assign parental lines in functional breeding programs.

Conclusion

In conclusion, this investigation has shown that domesticated and wild ecotypes of raspberry fruits are a rich source of p-coumaric acid and other phenolics. Also, lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits was found to be an effective antioxidant and radical scavenging activity in several *in vitro* assays including reducing power, $DPPH^{\bullet}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, and $O_2^{\bullet-}$ radical scavenging, H_2O_2 scavenging, and metal chelating activities as trolox equivalents. These assays have important applications for the food industry. Also, the results of this study show that the extract of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits can

Table 3–$DPPH^{\bullet}$, $ABTS^{\bullet+}$, $DMPD^{\bullet+}$, and $O_2^{\bullet-}$ radical scavenging, ferrous ion (Fe^{2+}) chelating, Fe^{3+}-Fe^{2+} reducing ability, Cu^{2+}-Cu^{+} reducing ability of lyophilized aqueous extract of raspberries fruits as trolox equivalent ($DPPH^{\bullet}$: 1,1-diphenyl-2-picryl-hydrazyl radicals, $ABTS^{\bullet+}$: 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radicals, $DMPD^{\bullet+}$: N,N-dimethyl-p-phenylenediamine radicals, $O_2^{\bullet-}$: superoxide anion radicals radicals).

	$DPPH^{\bullet}$ scavenging activities*	$ABTS^{\bullet+}$ scavenging activities*	$DMPD^{\bullet+}$ scavenging activities*	$O_2^{\bullet-}$ scavenging activities*	Fe^{2+} chelating activities*	Fe^{3+}-Fe^{2+} reducing activities*	Cu^{2+}-Cu^{+} reducing activities*
Wild Yayla ecotype	0.899 ± 0.056^{a}	0.934 ± 0.124^{a}	1.928 ± 0.125^{a}	3.884 ± 0.301^{a}	0.934 ± 0.121^{a}	0.658 ± 0.064^{a}	0.272 ± 0.011^{a}
Wild Yavuzlar ecotype	0.903 ± 0.121^{a}	1.007 ± 0.128^{a}	1.849 ± 0.184^{a}	3.296 ± 0.427^{b}	1.008 ± 0.213^{a}	0.507 ± 0.036^{b}	0.195 ± 0.024^{b}
Wild Yedigöl ecotype	0.617 ± 0.029^{b}	0.936 ± 0.098^{a}	2.065 ± 0.259^{b}	1.476 ± 0.126^{c}	0.937 ± 0.187^{a}	0.597 ± 0.049^{a}	0.221 ± 0.052^{b}
Willamette ecotype	0.628 ± 0.102^{b}	0.981 ± 0.067^{a}	1.634 ± 0.223^{c}	3.041 ± 0.251^{d}	0.981 ± 0.056^{a}	0.442 ± 0.061^{a}	0.144 ± 0.018^{c}

*Different superscripts (a, b and c) are used to show significant differences between parameters within each group ($p < 0.05$).

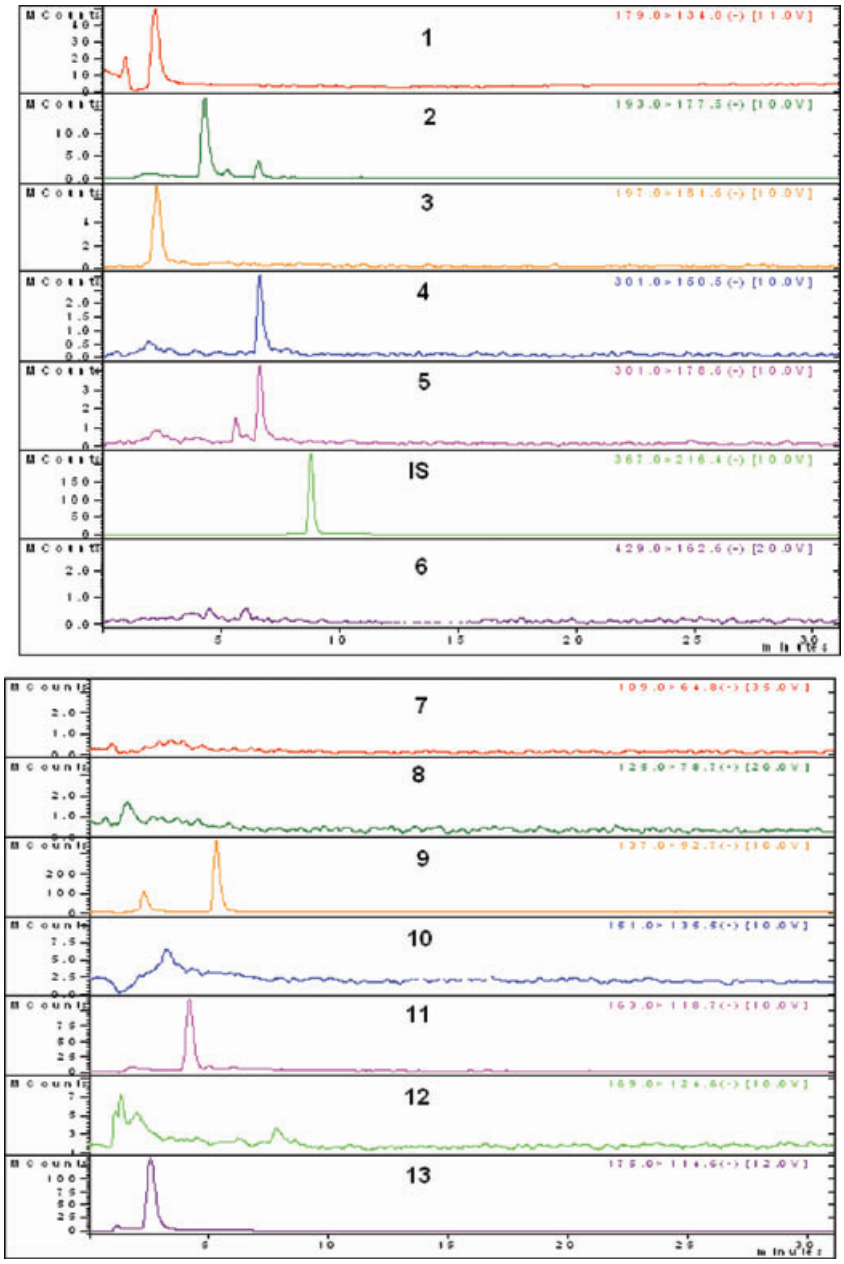

Figure 1–Chromatogram of antioxidants by LC-MS-MS of wild Yavuzlar ecotype. (Diluted sample chromatogram for the correct determination of 3–8 in the linear range of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits analysis.) Caffeic acid (1), ferulic acid (2), syringic acid (3), ellagic acid (4), quercetin (5), α-tocopherol (6), catechol (7), pyrogallol (8), p-hydroxybenzoic acid (9), vanillin (10), p-coumaric acid (11), gallic acid (12), ascorbic acid (13), and curcumin (IS).

be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. The results are clearly indicated that phenolic compounds appear to be responsible for the antioxidant activity of lyophilized aqueous extracts of domesticated and wild ecotypes of raspberry fruits.

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References

Ak T, Gülçin İ. 2008. Antioxidant and radical scavenging properties of curcumin. Chem Biol Interact 174:27–37.

Anttonen MJ, Karjalainen RO. 2005. Environmental and genetic variation of phenolic compounds in red raspberry. J Food Com Anal 18:759–69.

Apak R, Güçlü K, Özyürek M, Karademir SE, Erça E. 2006. The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. Int J Food Sci Nut 57:292–304.

[AOAC] Assoc. of Official Analytical Chemists Intl. 2005. Official methods of analysis, 18th ed. Gaithersburg, Md.: AOAC.

Beekwilder J, Jonker H, Meesters P, Hall RD, Van Der Meer IM, Ric de Vos CH. 2005. Antioxidants in raspberry: on-line analysis links antioxidant activity to a diversity of individual metabolites. J Agric Food Chem 53:3313–20.

Büyükokuroğlu ME, Gülçin İ, Oktay M, Kufrevioğlu ÖI. 2001. *In vitro* antioxidant properties of dantrolene sodium. Pharmacol Res 44:491–5.

Davis PH. 1982. Flora of Turkey and the east Aegean Islands. Vol. 4, Univ. Press of Edinburgh.

De Ancos B, González EM, Cano MP. 2000. Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. J Agric Food Chem 48:4565–70.

Dinis TCP, Madeira VMC, Almeida LM. 1994. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 315:161–9.

Eberhardt MV, Lee CY, Liu RH. 2000. Antioxidant activity of fresh apples. Nature 405:903–4.

Elmastas M, Türkekul İ, Öztürk L, Gülçin İ, Işıldak Ö, Aboul-Enein HY. 2006. The antioxidant activity of two wild edible mushrooms (*Morchella vulgaris* and *Morchella esculanta*). Comb Chem High T Scr 9:443–8.

EURACHEM/CITAC Guide, Quantifying uncertainty in analytical measurement. Second ed. 2000. London, U.K.

Eyduran SP, Agaoglu YS, Eyduran E, Ozdemir T. 2007. Comparison of some raspberry cultivars herbal features by repeated random complete design statistic technique. Pak J Biol Sci 10:1270–5.
Gören AC, Bilsel G, Bilsel M. 2007. Rapid and simultaneous determination of 25-OH-vitamin D_2 and D_3 in human serum by LC/MS/MS: validation and uncertainty assessment. J Chem Metrol 1:1–10.
Gören AC, Çıkrıkçı S, Çergel M, Bilsel G. 2009. Rapid quantitation of curcumin in turmeric via NMR and LC–tandem mass spectrometry. Food Chem 113:1239–42.
Gülçin İ. 2006a. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicology 217:213–20.
Gülçin İ. 2006b. Antioxidant and antiradical activities of L-Carnitine. Life Sci 78:803–11.
Gülçin İ. 2007. Comparison of in vitro antioxidant and antiradical activities of L-tyrosine and L-Dopa. Amino Acids 32:431–8.
Gülçin İ. 2008. Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent. J Enzym Inhib Med Chem 23:871–6.
Gülçin İ. 2010. Antioxidant properties of resveratrol: a structure-activity insight. Innov Food Sci Emerg 11:210–8.
Gülçin İ, Daştan A. 2007. Synthesis of dimeric phenol derivatives and determination of in vitro antioxidant and radical scavenging activities. J Enzym Inhib Med Chem 22:685–95.
Gülçin İ, Şat İG, Beydemir Ş, Elmastaş M, Küfrevioğlu Öİ. 2004. Comparison of antioxidant activity of clove (*Eugenia caryophylata* Thunb) buds and lavender (*Lavandula stoechas* L.). Food Chem 87:393–400.
Gülçin İ, Berashvili D, Gepdiremen A. 2005a. Antiradical and antioxidant activity of total anthocyanins from *Perilla pankinensis* decne. J Ethnopharmacol 101:287–93.
Gülçin İ, Beydemir Ş, Şat İG, Küfrevioğlu Öİ. 2005b. Evaluation of antioxidant activity of cornelian cherry (*Cornus mas* L.). Acta Aliment Hung 34:193–202.
Gülçin İ, Elias R, Gepdiremen A, Boyer L. 2006. Antioxidant activity of lignans from fringe tree (*Chionanthus virginicus* L.). Eur Food Res Technol 223:759–67.
Gülçin İ, Tel AZ, Kirecci E. 2008. Antioxidant, antimicrobial, antifungal and antiradical activities of *Cyclotrichium niveum* (Boiss.) Manden and Scheng. Int J Food Propert 11:450–71.
Gülçin İ, Bursal E, Şehitoğlu HM, Bilsel M, Gören AC. 2010a. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of propolis from Erzurum, Turkey. Food Chem Toxicol 48:2227–38.
Gülçin İ, Elias R, Gepdiremen A, Chea A, Topal F. 2010b. Antioxidant activity of bisbenzylisoquinoline alkaloids from *Stephania rotunda*: cepharanthine and fangchinoline. J Enzym Inhib Med Chem 25:44–53.
Gülçin İ, Kirecci E, Akkemik E, Topal F, Hisar O. 2010c. Antioxidant and antimicrobial activities of an aquatic plant: Duckweed (*Lemna minor* L.). Turk J Biol 34:175–88.
Isabelle M, Lee BL, Lim MT, Koh WP, Huang D, Ong CN. 2010. Antioxidant activity and profiles of common fruits in Singapore. Food Chem 123:77–84.
Kafkas E, Ozgen M, Ozogul Y, Turemis N. 2008. Phytochemical and fatty acid profile of selected red raspberry cultivars: a comparative study. J Food Qual 31:67–8.
Kahkonen MP, Hopia AI, Heinonen M. 2001. Berry phenolics and their antioxidant activity. J Agric Food Chem 49:4076–82.
Köksal E, Gülçin İ. 2008. Antioxidant activity of cauliflower (*Brassica oleracea* L.). Turk J Agric For 32:65–78.
Liu M, Li Q, Weber C, Lee CY, Brown J, Liu RH. 2002. Antioxidant and antiproliferative activities of raspberries. J Agric Food Chem 50:2926–30.
Mullen W, McGinn J, Lean MEJ, MacLean MR Gardner P, Duthie GG, Yokota T Crozier A. 2002. Ellagitannins, flavonoids, and other phenolics in red raspberries and their contribution to antioxidant capacity and vasorelaxation properties. J Agric Food Chem 50:5191–6.
Pantelidis GE, Vasilakakis M, Manganaris GA, Diamantidis GR. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. Food Chem 102:777–83.
Ryabova D. 2007. Population evaluation in crop wild relatives for in situ conservation: a case study for raspberry *Rubus idaeus* L. in the Leningrad region, Russia. Genetic Resources and Crop Evolution 54:973–80.
Singleton VL, Rossi JL. 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Viticult 16:144–8.
Stewart D, McDougall GJ, Sungurtas J, Verrall S, Graham J, Martinussen I. 2007. Metabolomic approach to identifying bioactive compounds in berries: advances toward fruit nutritional enhancement. Mol Nutr Food Res 51:645–51.
Viskelis P, Rubinskiene M, Buskiene L, Bobinaite R. 2006. Quality of raspberries grown in Lithuania. Sodininkyste ir Darzininkyste 25:74–80.
Wang SY, Lin HS. 2000. Antioxidant activity in fruit and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. J Agric Food Chem 48:140–6.
Woodhead M, McCallum S, Smith K, Cardle L, Mazzitelli L, Graham J. 2008. Identification, characterization and mapping of simple sequence repeat (SSR) markers from raspberry root and bud ESTs. Mol Breed 22:555–63.