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CHEMICAL AND ANTIOXIDANT PROPERTIES OF CULTIVATED AND WILD *FRAGARIA* AND *RUBUS* BERRIES

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ABSTRACT

Content of individual sugars, organic acids, total phenolics (TPH), some important phenolic compounds (kaempferol, myricetin, quercetin, and ellagic acid) and total antioxidant capacity (TAC) in the fruits of strawberry, raspberry and blackberry were studied. A comparison was made between cultivars and wild relatives of each species (*Fragaria vesca* L., *Rubus idaeus* L. and *Rubus fruticosus* L.). The main sugars found were fructose and glucose both in the fruits of wild species and the studied cultivars. Citric acid was determined to be the major organic acid in most of tested berries with the exception of blackberry cultivars, where malic acid was dominant. The content of individual phenolic compounds varied among the wild species, as well as among the studied cultivars. Ellagic acid content was higher in *F. vesca*, *R. idaeus* and *R. fruticosus* (122.5 μg/g FW, 12.71 μg/g FW and 61.7 μg/g FW, respectively) than that obtained in analyzed cultivars. Overall, TPH expressed higher values in the wild strawberry and blackberry species in comparison to the studied cultivars, and consequently, the highest levels of TAC were recorded in *F. vesca* (5.78 mg asc/g FW), followed by *R. fruticosus* (4.95 mg asc/g FW).

PRACTICAL APPLICATIONS

In recent years, both wild and cultivated berries have become very attractive for consumers because of potentially beneficial phytochemicals contained in these fruits. Fruit nutritional quality can be described by standard quality parameters (sugars and organic acids), and the analysis of antioxidant capacity influenced by specific related compounds. The importance of flavonoids and other phenolics have been suggested to play a preventive role in the development of cancer and heart disease. A significant positive correlation observed in this study between total phenolics and total antioxidant capacity indicate the need for the use of wild species in the breeding programs of small fruits, especially strawberry and blackberry, in order to increase their nutritive value and the health benefits.

INTRODUCTION

Berries are widely recognized fruits for their nutritional quality and potential health benefits. Several studies have shown that these fruits generally possess high levels of sugars and organic acids determining their sweetness and acidity (Sturm *et al.* 2003; Kafkas *et al.* 2006). Since fruit taste depends not only on the total sugar and organic acid contents but also on the type and the quantity of individual compounds, their composition may reflect changes in fruit quality.

In past decades, pronounced attention has been paid to the antioxidant capacity of fruit as an eligible parameter of quality (Vangdal and Slimestad 2006). This parameter is strictly correlated to the presence of efficient oxygen radical scavengers, such as vitamin C and phenolic compounds, which have been shown to play an important role in controlling oxidative reactions in the human body and exhibit anticarcinogenic activities (Wang and Jiao 2000; Sun *et al.* 2002; Scalzo *et al.* 2005; Tulipani *et al.* 2008). Flavonoids and phenolic acids are the most common phenolic compounds in small fruits with strong antioxidant capacity (Wang and Lin 2000; Meyers *et al.* 2003; Cho *et al.* 2005). Their content in fruits varies among species and cultivar, but it can also be affected by growth conditions including environmental factors and cultivation techniques (Dixon and Paiva 1995; Deighton *et al.* 2000; Moyer *et al.* 2002; Mullen *et al.* 2002; Määttä-Riihinen *et al.* 2004; Scalzo *et al.* 2005).

Phenolic acids constitute about one-third of the dietary phenols (Robbins 2003; Zadernowski *et al.* 2005), and strawberries, raspberries and blackberries are considered to be among the fruits with considerable amount of ellagic acid (Daniel *et al.* 1989; Clifford and Scalbert 2000; Koponen *et al.* 2007). Also, these berries are known for their content of flavonols such as quercetin, kaempferol and myricetin as well as their derivatives (primarily glycosides) which may provide health benefits as dietary antioxidants (Hollman and Katan 1999; Siriwoharn and Wrolstad 2004).

Taking into account the importance of the antioxidant capacity exhibited by flavonoids and phenolic acids, the purpose of this study was to determine the phenolic profiles in fruits of three *Fragaria* and *Rubus* species and to compare them to those in the major cultivars in Serbia. The variability in the content and composition of identified phenolic compounds, as well as the correlations between total phenolic (TPH) content and antioxidant capacity, can point to genetic differences among wild species and cultivated varieties of these berries. It may provide a better understanding on the wild species' role as an important native genetic source for breeding new cultivars with high levels of phenolic compounds, contributing to both better sensorial attributes and their antioxidant capacity. Potential differences in expressed antioxidant capacity of fruit among cultivars of the analyzed berries will indicate the importance of improving the structure of the assortment in commercial plantings. It means that by the introduction of cultivars, which apart from high productivity and attractive fruit appearance possess high nutritive and antioxidant values, it is possible to increase fruit consumption for beneficial health purposes without decreasing the commercial effect of production.

MATERIALS AND METHODS

Plant Material

Ripe fruits of three berry species, *Fragaria vesca* L., *Rubus idaeus* L. and *Rubus fruticosus* L., were harvested from native populations in Western Serbia (municipality of Dragačevo) during a 3-year period (2004–2006) and analyzed. They were compared to ripe fruits of two strawberry cultivars of *F.* × *ananassa* Duch. ("Marmolada" and "Madeleine"), two raspberry cultivars ("Wilamette" and "Meeker") and two thornless blackberry cultivars ("Thornfree" and "Čačanska bestrna"). Fruits of selected cultivars were harvested at the commercial plantation located in the same region as the studied wild species.

All samples were extracted immediately after harvesting. Approximately 100 g of fruits were pureed, and samples of 5 g were homogenized for 1 min in 20 mL of extraction solution containing methanol/water/hydrochloric acid at a ratio of 70:30:5 by volume. The homogenate was filtered through a filter paper and the filtrates were centrifuged at 3,000 × *g* for 15 min. The methanol supernatant was divided into aliquots and frozen at −80C until analysis. Triplicate extractions were prepared for each fruit analyzed.

All chemicals and solvents were purchased from Sigma Chemical Company (Sigma–Aldrich, Oakville, Canada) and for all analysis 18 MΩ deionized water was used (Millipore, Bedford, MA).

Analytical Procedures

Determination of Individual Sugars. Separations were performed on a Waters Breeze chromatographic system (Waters, Milford, MA) containing 1,525 binary pumps system, thermostated column compartment and 2,465 Waters electrochemical detector, equipped with gold working electrode and hydrogen referent electrode. Separation of sugars was performed on CarboPac PA1 (Dionex, Sunnyvale, CA) 250 × 4 mm column equipped with corresponding CarboPac PA1 guard column. Sugars were eluted with 200 mM NaOH for 20 min at a flow rate of 1.0 mL/min at constant temperature of 30C. Signals were detected in a pulse mode with the following waveform: $E_1 = +0.1$ V for 280 ms; $E_2 = +0.75$ V for 150 ms; $E_3 = -0.85$ V for 150 ms and within 80 ms of integration time. Filter timescale was 0.2 s and range was 200 to 500 nA for the full mV scale. Data acquisition and evaluation were carried out by Waters Empower 2 software.

Determination of Organic Acids. Separation of organic acids was performed on Hewlett Packard HP1100 series chromatograph (Palo Alto, CA) composed of inline degasser, autosampler, thermostated compartment and HP1100 Photo Diode Array detector adjusted at 210 nm, with reference signal at 600 nm. An anion exchange column (Aminex HPX-87H, Bio-Rad Lab., CA) 300 × 7.8 mm was used with 5 mM H_2SO_4 as mobile phase. Elution used was isocratic with a flow rate of 0.6 mL/min at 40C. Data acquisition and evaluation were carried out by Agilent Chemstation software (Wilmington, DE).

Determination of Flavonols and Ellagic Acid Content. Quantification of individual phenolic compounds was done by reversed phase high-performance liquid chro-

TABLE 1. INDIVIDUAL SUGAR AND ORGANIC ACID CONTENTS IN STRAWBERRY FRUITS (mg/g FW)

Wild species/Cultivar	Sugars			Organic acids	
	Glucose	Fructose	Sucrose	Citric	Malic
Fragaria vesca	65.2 ± 1.35a	116.8 ± 2.04a	27.4 ± 1.64a	0.21 ± 0.02a	0.10 ± 0.01a
Marmolada	45.2 ± 2.05b	58.8 ± 3.34b	5.1 ± 1.36b	0.07 ± 0.00b	0.03 ± 0.00b
Madeleine	45.8 ± 1.92b	62.1 ± 3.75b	3.7 ± 0.42b	0.08 ± 0.01b	0.02 ± 0.00b

Mean of 3-year values with three replications in every year. Values are mean ± standard error. For each parameter, different letters indicate significant differences at $P \leq 0.05$ between species or cultivars.
FW, fresh weight.

matography (HPLC) analysis. Samples were injected in Waters HPLC system consisted of 1525 binary pumps, thermostat and 717+ autosampler connected to the Waters 2996 diode array detector (Waters, Milford, MA). Chromatograms were gathered in 3D mode with extracted signals at specific wavelengths for different compounds (370, 326 and 254 nm, respectively). Separation of phenolics was performed on a Symmetry C-18 RP column 125 × 4 mm size with 5 μm particle diameter (Waters) connected to appropriate guard column. Two mobile phases, A (0.1% phosphoric acid) and B (acetonitrile), were used at a flow of 1 mL/min with the following gradient profile: the first 20 min from 10 to 22% B; next 20 min of linear rise up to 40% B, followed by 5 min reverse to 10% B and additional 7.5 min of equilibration time. The data acquisition and spectral evaluation for peak confirmation were carried out by the Waters Empower 2 Software (Waters).

Determination of Total Phenolics (TPH). The method employed was based on Folin–Ciocalteau's phenol reagent and spectrophotometric determination (Singleton and Rossi 1965). Results were expressed as milligrams of gallic acid equivalent per a gram of fresh weight (mg GAE/g FW).

Determination of the Total Antioxidant Capacity. Total antioxidant capacity (TAC) was measured by using the ABTS method according to Arnao *et al.* (1999). The reaction mixture contained 2 mM ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), 15 μM hydrogen peroxide, 0.25 μM HRP and 20 μL of 80% methanol extract of the powdered fruits in 50 mM phosphate buffer pH 7.5 in a total volume of 2 mL. The assay temperature was 25C. The absorbance of the reaction mixture at 730 nm was determined using 2501 PC Shimadzu UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). The reaction was monitored until a stable absorbance was obtained due to ABTS radical formation. Ascorbic acid was used as a standard. Results were expressed as milligrams of ascorbic acid equivalent per a gram of fresh weight (mg asc/g FW).

Statistical Analysis

Statistical analysis was performed using software Statistica 6.0 for Windows (StatSoft Inc., Tulsa, OK). Data from a 3-year investigation were calculated by multivariate analysis of variance for mean comparison, and intergenotype significance of differences was calculated according to least significant difference test. Data are reported as means ± standard error of the mean. Correlations between TAC and TPH were calculated separately for each species, according to Pearson's test. Differences at $P \leq 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Sugars and Organic Acids Content

Individual sugar and organic acid contents in strawberry fruits are presented in Table 1. The highest average contents of glucose, fructose and sucrose were obtained in *F. vesca* (65.2 mg/g FW, 116.8 mg/g FW, and 27.4 mg/g FW, respectively). The main sugars were fructose and glucose in all strawberry fruits. Sucrose was detected in small quantities, particularly in the fruits of "Marmolada" (5.1 mg/g FW) and "Madeleine" (3.7 mg/g FW). Lower amounts of individual sugars were reported by Sturm *et al.* (2003) for "Marmolada" and Faedi *et al.* (2004) for "Madeleine." The obtained data confirm that numerous factors can influence the chemical fruit composition, such as maturity stage, ecological conditions, irrigation and fertilization. The organic acids content in strawberries was similar in studied cultivars, but higher in wild strawberry. The main organic acid was citric acid, twice as low quantities of malic acid being observed in all strawberries tested.

Sugars and organic acid contents in raspberries varied among cultivars and wild species (Table 2). Among the sugars detected in raspberry fruits, highest average amount of glucose was detected in *R. idaeus* (38.3 mg/g FW). On the other hand, higher levels of fructose were obtained in the fruits of studied cultivars. The sucrose content, which

TABLE 2. INDIVIDUAL SUGAR AND ORGANIC ACID CONTENTS IN RASPBERRY FRUITS (mg/g FW)

Wild species/Cultivar	Sugars			Organic acids	
	Glucose	Fructose	Sucrose	Citric	Malic
Rubus idaeus	38.3 ± 1.33a	31.5 ± 1.14b	6.9 ± 0.53a	0.15 ± 0.01c	0.08 ± 0.01a
Willamette	35.2 ± 3.93a	46.8 ± 4.01a	6.4 ± 0.65a	0.24 ± 0.02a	0.05 ± 0.01b
Meeker	36.2 ± 2.22a	49.2 ± 2.81a	5.3 ± 0.58b	0.18 ± 0.01b	0.04 ± 0.00b

Mean of 3-year values with three replications in every year. Values are mean ± standard error. For each parameter, different letters indicate significant differences at $P \leq 0.05$ between species or cultivars.
FW, fresh weight.

presents a small share among the sugars, ranged from 5.3 mg/g FW ("Meeker") to 6.9 mg/g FW (*R. idaeus*). Kafkas *et al*. (2008) detected higher content of sucrose in seven raspberry cultivars with the highest value recorded in cultivar "Willamette" (16.15 g/kg FW). Higher sugar content does not automatically mean sweeter–tasting raspberries, since the amount of organic acids is also important in the perception of fruit taste. The main organic acid in raspberries was citric acid, significant amounts was detected in fruits of cultivar "Willamette" (0.24 mg/g FW), demonstrating the superior quality of this cultivar for processing. The lowest content of citric acid was found in *R. idaeus* (0.15 mg/g FW), which in turn exhibited the highest content of malic acid (0.08 mg/g FW).

The composition of sugars and organic acids detected in blackberry fruits are given in Table 3. The presence of fructose was predominant in all studied blackberry samples, followed by glucose. This is in accordance with previously published data (Kafkas *et al*. 2006). In this study, *R. fruticosus* expressed the lowest values of glucose, fructose and sucrose contents (76.1 mg/g FW, 64.5 mg/g FW, 3.0 mg/g FW, respectively). The highest levels of fructose and glucose were recorded in cultivar "Thornfree." Sucrose was present in much lower quantities compared to the other sugars in cultivars and wild relatives due to the fact that it may be converted to inverted forms during the ripening process. Malic acid was found to be dominant in studied cultivars, whereas citric acid was the main acid detected in *R. fruticosus*. Significant differences in malic acid content were observed between cultivars and wild species. In research on different blackberry cultivars by Kafkas *et al*. (2006), citric acid was not detected in any of investigated cultivars, but the quantities of malic acid were similar to those obtained in this study.

Flavonols and Ellagic Acid Content

The contents of individual flavonols and ellagic acid in strawberry fruits are presented in Table 4. It was observed that *F. vesca* and both of the studied cultivars contained similar quantities of kaempferol. The myricetin content ranged from

TABLE 3. INDIVIDUAL SUGAR AND ORGANIC ACID CONTENTS IN BLACKBERRY FRUITS (mg/g FW)

Wild species/Cultivar	Sugars			Organic acids	
	Glucose	Fructose	Sucrose	Citric	Malic
Rubus fruticosus	64.5 ± 2.74a	76.1 ± 2.18b	3.0 ± 0.42b	0.10 ± 0.01a	0.03 ± 0.01c
Thornfree	67.7 ± 5.65a	88.1 ± 5.68a	3.1 ± 0.64b	0.10 ± 0.01a	0.17 ± 0.02a
Čačanska bestrna	66.8 ± 4.38a	86.0 ± 6.11a	4.2 ± 1.00a	0.09 ± 0.01a	0.11 ± 0.01b

Mean of 3-year values with three replications in every year. Values are mean ± standard error. For each parameter different letters indicate significant differences at $P \leq 0.05$ between species or cultivars.
FW, fresh weight.

TABLE 4. FLAVONOLS AND ELLAGIC ACID CONTENTS IN STRAWBERRY FRUITS (μg/g FW)

Wild species/Cultivar	Flavonols			
	Kaempferol	Myricetin	Quercetin	Ellagic acid
Fragaria vesca	2.35 ± 0.38a	0.50 ± 0.04c	7.34 ± 0.24a	122.5 ± 15.9a
Marmolada	2.89 ± 0.19a	1.04 ± 0.19b	1.79 ± 0.15b	21.3 ± 3.67c
Madeleine	2.35 ± 0.58a	1.26 ± 0.14a	1.60 ± 0.05b	32.2 ± 6.65b

Mean of 3-year values with three replications in every year. Values are mean ± standard error. For each parameter different letters indicate significant differences at $P \leq 0.05$ between species or cultivars.
FW, fresh weight.

TABLE 5. FLAVONOLS AND ELLAGIC ACID CONTENTS IN RASPBERRY FRUITS (μg/g FW)

Wild species/Cultivar	Flavonols			Ellagic acid
	Kaempferol	Myricetin	Quercetin	
Rubus idaeus	0.13 ± 0.02b	0.25 ± 0.04a	ND	12.71 ± 1.54a
Willamette	2.40 ± 0.22a	0.26 ± 0.08a	0.71 ± 0.01	1.98 ± 0.15c
Meeker	0.40 ± 0.09b	0.31 ± 0.07a	ND	5.24 ± 1.82b

Mean of 3-year values with three replications in every year. Values are mean ± standard error. For each parameter different letters indicate significant differences at $P \leq 0.05$ between species or cultivars.
FW, fresh weight; ND, not detectable.

0.50 μg/g FW for *F. vesca* to 1.26 μg/g FW for "Madeleine." In general, the amounts of myricetin were two to four times lower than those of kaempferol. Quercetin was predominant flavonol in *F. vesca* (7.34 μg/g FW), whereas lower and quite similar values were obtained in studied cultivars. These results are comparable with the findings previously reported (Häkkinen *et al.* 1999; Cordenunsi *et al.* 2005; Tulipani *et al.* 2008). Although flavonols are present in minor quantities in strawberry fruits, phenolic acids proved to be of interest, in particular ellagic acid, which represents one of the main phenolic compounds in these fruits (Daniel *et al.* 1989; Amakura *et al.* 2000; Määttä-Riihinen *et al.* 2004; Aaby *et al.* 2005; Koponen *et al.* 2007).

Large differences in free ellagic acid content were observed between *F. vesca* and cultivars, achieving the highest value in wild strawberry (122.5 μg/g FW). Also, significant difference was observed between the two studied cultivars. Maas *et al.* (1991) and Cordenunsi *et al.* (2005) noticed variability in ellagic acid content in fruits of different strawberry cultivars.

The obtained results of the individual flavonol content in tested raspberry fruit indicated that the wild species and cultivars had similar values of myricetin content (Table 5). Quercetin was only detected in the fruits of cultivar "Willamette" (0.71 μg/g FW), which is also characterized by the highest amount of kaempferol (2.40 μg/g FW). As opposed to our results, Justesen *et al.* (1998) and Häkkinen *et al.* (1999) observed higher quantities of quercetin in raspberry fruits.

Relatively high content of free ellagic acid was detected in *R. idaeus* (12.71 μg/g FW), as expected. The lowest average value of ellagic acid content was obtained in cultivar "Willamette" (1.98 μg/g FW), which had the highest flavonols content. In general, free ellagic acid levels observed in raspberry are quite low in this study, and their detection is probably the result of acid hydrolysis products of ellagitannin breakdown (Mullen *et al.* 2002).

The amounts of individual flavonols and ellagic acid were significantly different between wild blackberry and common commercial cultivars (Table 6). *R. fruticosus* expressed highest values of kaempferol, myricetin and ellagic acid contents (2.76 μg/g FW, 3.14 μg/g FW, 61.7 μg/g FW, respectively). In accordance with the earlier finding for ellagic acid content in Georgia-grown blackberries (Sellappan *et al.* 2002), significant quantities of ellagic acid were also detected in analyzed blackberry cultivars, but these are twice as low than that recorded in *R. fruticosus*. This discrepancy is most likely due to genetic differences, since the fruits of *R. fruticosus* were taken from native populations in Western Serbia, whereas the studied cultivars were derived as results of hybridization.

Quercetin was not detected in any of the blackberry samples tested in this study, which indicates that quercetin derivatives were predominately present in blackberries. Several studies have already reported the presence of quercetin glycosides in blackberries (Henning 1981; Bilyk and Sapers 1986; Siriwoharn *et al.* 2004).

Berries are one of the main sources of ellagic acid which is normally present as a polymer (ellagitannin) or glycosylated derivative (Häkkinen *et al.* 1999; Siriwoharn *et al.* 2004; Cordenunsi *et al.* 2005). The obtained results of ellagic acid content in this study indicated that ellagitannins present in strawberries could be more easily decomposed and converted to free ellagic acid than in raspberry fruits, which was shown

TABLE 6. FLAVONOLS AND ELLAGIC ACID CONTENTS IN BLACKBERRY FRUITS (μg/g FW)

Wild species/Cultivar	Flavonols			Ellagic acid
	Kaempferol	Myricetin	Quercetin	
Rubus fruticosus	2.76 ± 0.26a	3.14 ± 0.21a	ND	61.7 ± 3.70a
Thornfree	1.88 ± 0.21b	1.02 ± 0.24b	ND	35.5 ± 1.23b
Čačanska bestrna	1.55 ± 0.31b	0.31 ± 0.08c	ND	30.9 ± 1.83c

Mean of 3-year values with three replications in every year. Values are mean ± standard error. For each parameter different letters indicate significant differences at $P \leq 0.05$ between species or cultivars.
FW, fresh weight; ND, not detectable.

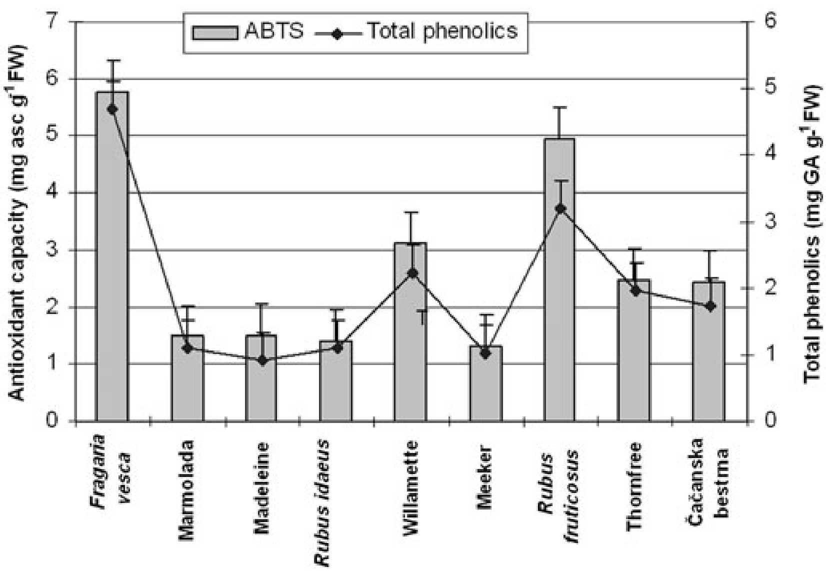

FIG. 1. SURVEY OF ANTIOXIDANT CAPACITY (ABTS) AND TOTAL PHENOLIC CONTENT IN CULTIVATED AND WILD *FRAGARIA* AND *RUBUS* BERRIES
Data are mean ± standard error. FW is fresh weight.

by earlier findings (Häkkinen *et al.* 2000; Koponen *et al.* 2007). Besides, our results clearly show that flavonols constituted a minor proportion of TPH in blackberries, whereas proportion of ellagic acid was much higher as reported before (Sellappan *et al.* 2002; Cho *et al.* 2005).

Total Antioxidant Capacity and TPH

Since the antioxidant capacity of individual phenolic compounds cannot always be evaluated because of potential interactions among them (Pinelo *et al.* 2004), the determination of the TAC allows a more realistic evaluation of the protective effects of the analyzed fruit (Fig. 1).

In this study, TAC varied among three analyzed wild species, as well as among wild species and studied cultivars within each species. The highest values of TAC were recorded in *F. vesca* (5.78 mg asc/g FW), followed by *R. fruticosus* (4.95 mg asc/g FW). The commercial strawberry and blackberry cultivars are characterized by much lower TAC than their wild relatives. Such results confirm the need to evaluate the diversity of native populations of these species and, based on this, well-focused breeding programs can create new cultivars specifically selected for improved antioxidant potential.

No significant differences of TAC were observed between strawberry cultivars ("Marmolada" and "Madeleine"), nor between blackberry cultivars ("Thornfree" and "Čačanska bestrna"), probably reflecting similar expressed TPH.

Concerning raspberries, the most widely grown cultivar in Serbia, "Willamette," possessed higher TAC than those for *R. idaeus* (1.41 mg asc/g FW) and "Meeker" cultivar (1.32 mg asc/g FW). It was observed that TPH followed the similar trend as that of TAC in all studied berries (Fig. 1), with the highest values in the fruits of wild species *F. vesca* and *R. fruticosus* (4.69 mg GAE/g FW and 3.20 mg GAE/g FW, respectively). These data underline the importance of phenolic compounds in expressed antioxidant capacity, as reported by Proteggente *et al.* (2002) and Scalzo *et al.* (2005).

The quantity of TPH obtained in this study for *R. fruticosus* is generally in good agreement with previously published data (Rotundo *et al.* 1998), whereas the blackberry cultivars expressed much lower values than *R. fruticosus* ranging from 1.74 mg GAE/g FW ("Čačanska bestrna") to 1.97 mg GAE/g FW ("Thornfree"). These results are slightly lower than those obtained in some thornless blackberry cultivars grown in Italy (Benvenuti *et al.* 2004). The mean contents of TPH measured in analyzed raspberry fruits are in accordance with those reported by Ancos *et al.* (2000).

We have also calculated the correlation coefficients between TAC and TPH in all examined wild species and cultivars (Table 7). A very significant correlation between TAC and TPH was found in *R. fruticosus*, raspberry cultivar "Willamette," and blackberry cultivar "Čačanska bestrna" ($r = 0.82$, $r = 0.97$, and $r = 0.98$, respectively), confirming the previously established finding for several species of berries (Deighton *et al.* 2000).

As noted before (Pinelo *et al.* 2004; Tulipani *et al.* 2008), significant correlation, observed between TAC and TPH in this study, confirms the possibility of using the parameter "total phenolics" as an indicator of antioxidant capacity. However, the contribution of individual phenolics to expressed antioxidant capacity is still poorly defined.

No statistically significant correlation could be observed in strawberry cultivars, indicating that some other compounds, such as vitamin C, make an important contribution to the expressed TAC. This is in line with the previous findings of Kähkönen *et al.* (1999) and Scalzo *et al.* (2005).

TABLE 7. PEARSON'S CORRELATION COEFFICIENTS (R_{XY}) BETWEEN TAC AND TPH IN CULTIVATED AND WILD *FRAGARIA* AND *RUBUS* BERRIES

Species/Cultivar	Coefficients (rxy)
Strawberry	
Fragaria vesca L.	0.78*
Marmolada	0.62^{ns}
Madeleine	0.52^{ns}
Raspberry	
Rubus idaeus L.	0.70*
Willamette	0.97**
Meeker	0.89*
Blackberry	
Rubus fruticosus L.	0.82**
Thornfree	0.76*
Čačanska bestrna	0.98**

* Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$.
NS, nonsignificant.

The results of all studied individual phenolics, TPH and TAC represent 3-year average values. We observed differences in the content of analyzed individual phenolics between different years (data not shown). The content of kaempferol in raspberry and blackberry was the only parameter that was stable during the 3 years of investigation. Highest values of TPH and TAC were registered in the second year of investigation in all of the wild species and cultivars studied. Seasonal variations observed in the level of important phenolic compounds and therefore expressed antioxidant capacity can be explained by the influence of ecological factors (light, temperature, and rainfall) and cultural practices as reported before (Dixon and Paiva 1995; Wang and Zheng 2001; Halvorsen *et al.* 2002).

CONCLUSIONS

The results obtained in this study could be of interest for better definition of the breeding strategies, and confirm the importance of the genetic background in small fruits for the availability of specific compounds with high phytonutrient profiles. In the case of the sugar content, studied raspberry and blackberry cultivars produced higher quantities than their wild relatives. As opposed to raspberries and blackberries, the content of all the analyzed sugars was significantly higher in *F. vesca* than those found in the strawberry cultivars. Also, most of the analyzed phenolic compounds contained in *F. vesca* and *R. fruticosus* point out that wild species can contribute to the improvement of nutritional quality in cultivars through the selection of genotypes from native populations. This would mean that through the introduction of wild strawberry and blackberry species to breeding programs, a powerful tool for modifying the composition and content of important phenolic compounds could be obtained. The exception is *R. idaeus* due to lower values of TPH content and antioxidant capacity in comparison to "Willamette" cultivar. This indicates that new raspberry cultivars with high phenolic content can be obtained through the classical breeding techniques such as hybridization and clonal selection. Furthermore, these data are interesting because previous studies do not indicate such relevant differences among wild species of *Fragaria* and *Rubus* genera, and they are even more remarkable among the wild species and cultivars of each berries tested. It is important to underline that the strawberry, raspberry and blackberry cultivars in this study have all been grown in similar environmental conditions as their wild species, thus eliminating the influence of environmental factors in the expressed differences within the species.

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REFERENCES

AABY, K., SKREDE, G. and WROLSTAD, E.R. 2005. Phenolic composition and antioxidant activities in flesh and achenes of strawberries (*Fragaria ananassa*). J. Agric. Food Chem. *53*, 4032–4040.

AMAKURA, Y., OKADA, M., TSUJI, S. and TONOGAI, Y. 2000. High-performance liquid chromatographic determination with photo-diode array detection of ellagic acid in fresh and processed fruits. J. Chromatogr. A *896*, 87–93.

ANCOS, B., GONZÁLES, E.M. and CANO, M.P. 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*, 4565–4570.

ARNAO, M.B., CANO, A. and ACOSTA, M. 1999. Methods to measure the antioxidant activity in plant material. A comparative discussion. Free Radic. Res. *32*, 89–96.

BENVENUTI, S., PELLATI, F., MELEGARI, M. and BERTELLI, D. 2004. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus, Ribes* and *Aronia*. J. Food Sci. *69*, 164–169.

BILYK, A. and SAPERS, G.M. 1986. Varietal differences in the quercetin, kaempferol, and myricetin contents of highbush blueberry, cranberry, and thornless blackberry fruits. J. Agric. Food Chem. *34*, 585–588.

CHO, J.M., HOWARD, R.L., PRIOR, L.R. and CLARK, R.J. 2005. Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry. J. Agric. Food Chem. *85*, 2149–2158.

CLIFFORD, M.N. and SCALBERT, A. 2000. Ellagitannins – nature, occurrence and dietary burden. J. Sci. Food Agric. *80*, 1118–1125.

CORDENUNSI, B.R., GENOVESE, M.I., JOÄO, R.O., HASSIMOTTO, N.A., SANTOS, R.J. and LAJOLO, F.M. 2005. Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. Food Chem. *91*, 113–121.

DANIEL, E.M., KRUPNICK, A.S., HEUR, Y.H., BLINZLER, J.A., NIMS, R.W. and STONER, G.D. 1989. Extraction, stability, and quantification of ellagic acid in various fruits and nuts. J. Food Compost. Anal. *2*, 338–349.

DEIGHTON, N., BRENNAN, R., FINN, C. and DAVIES, H.V. 2000. Antioxidant properties of domesticated and wild *Rubus* species. J. Sci. Food Agric. *80*, 1307–1313.

DIXON, R.A. and PAIVA, N.L. 1995. Stress-induced phenylpropanoid metabolism. Plant Cell. *7*, 1085–1097.

FAEDI, W., BARUZZI, G., MALTONI, M.L., MAGNANI, S., MANZECCHI, L., LUCCHI, P., SBRIGHI, P. and TURCI, P. 2004. Nuove varieta e selezioni di fragola sperimentate in Romagna. Frutticoltura. *LXVI*, 30–38.

HÄKKINEN, S.H., KÄRENLAMPI, S.O., HEINONEN, I.M., MYKKÄNEN, M.H. and TÖRRÖNEN, A.R. 1999. Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. J. Agric. Food Chem. *47*, 2274–2279.

HÄKKINEN, S.H., KÄRENLAMPI, S.O., MYKKÄNEN, H.M., HEINONEN, J.M. and TÖRRÖNEN, A.R. 2000. Elagic acids content in berries: Influence of domestic processing and storage. Eur. Food Res. Technol. *212*, 75–80.

HALVORSEN, B.L., HOLTE, K., MYHRSTAD, M.C.W., BARIKMO, I., HVATTUM, E. and FAGERTUN, R.S. 2002. A systematic screening of total antioxidants in dietary plants. J. Nutr. *132*, 461–471.

HENNING, W. 1981. Flavonol glycosides of strawberries (*Fragaria* x *ananassa* Duch.), raspberries (*Rubus idaeus* L.) and blackberries (*Rubus fruticosus* L.). Phenolics of fruits. Z. Lebensm. Forsch. *173*, 180–187.

HOLLMAN, P.C.H. and KATAN, M.B. 1999. Health effects and bioavailability of dietary flavonols. Free Radic. Res. *31*, 75–80.

JUSTESEN, U., KNUTHSEN, P. and LETH, T. 1998. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high performance liquid chromatography with photo-diode array and mass spectrometric detection. J. Chromatogr. A *799*, 101–110.

KAFKAS, E., KOSAR, M., TŰREMIS, N. and BASER, K.H.C. 2006. Analysis of sugars, organic acids and vitamin C contents of blackberry genotypes from Turkey. Food Chem. *97*, 732–736.

KAFKAS, E., ÖZGEN, M., ÖZOĞUL, Y. and TŰREMIS, N. 2008. Phytochemical and fatty acid profile of selected red raspberry cultivars: A comparative study. J. Food Qual. *31*, 67–78.

KÄHKÖNEN, M.P., HOPIA, A.I., VUORELA, H.J., RAUHA, J.P., PIHLAJA, K., KUJALA, T.S. and HEINONEN, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem. *47*, 3954–3962.

KOPONEN, J.M., HAPPONEN, A.M., MATTILA, P.H. and TÖRRÖNEN, A.R. 2007. Contents of anthocyanins and ellagitannins in selected foods consumed in Finland. J. Agric. Food Chem. *65*, 1612–1619.

MAAS, J.L., WANG, S.Y. and GALLETTA, G.J. 1991. Evaluation of strawberry cultivars for ellagic acid content. HortScience. *26*, 66–68.

MÄÄTTÄ-RIIHINEN, K.R., KAMAL-ELDIN, A. and TÖRRÖNEN, A.R. 2004. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family *Rosaceae*). J. Agric. Food Chem. *52*, 6178–6187.

MEYERS, K.J., WATKINS, C.B., PRITTS, M.P. and LIU, R.H. 2003. Antioxidant and antiproliferative activities of strawberries. J. Agric. Food Chem. *51*, 6887–6892.

MOYER, R., HUMMER, K., WROLSTAD, R.E. and FINN, C. 2002. Antioxidant compounds in diverse *Ribes* and *Rubus* germplasm. Acta Hortic. *585*, 501–505.

MULLEN, W., MCGINN, J., LEAN, M.E.J., MACLEAN, M.R., GARDNER, P., DUTHIE, G.G., YOKOTA, T. and 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*, 5191–5196.

PINELO, M., MANZOCCO, L., NUNEZ, M.J. and NICOLI, M.C. 2004. Interaction among phenols in food fortification: Negative synergism on antioxidant capacity. J. Agric. Food Chem. *52*, 1177–1180.

PROTEGGENTE, A.R., PANNALA, A.S., PAGANGA, G., VAN BUREN, L., WAGNER, E., WISEMAN, S., VAN DE PUT, F., DACOMBE, C. and RICE-EVANS, C.A. 2002. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. Free Radic. Res. *36*, 217–233.

ROBBINS, R.J. 2003. Phenolic acids in foods: An overview of analytical methodology. J. Agric. Food Chem. *51*, 2866–2887.

ROTUNDO, A., BOUNOUS, G., BENVENUTI, S., VAMPA, G., MELEGARI, M. and SORAGNI, F. 1998. Quality and yield of *Ribes* and *Rubus* cultivars grown in Southern Italy hilly location. Phytother. Res. *12*, S135–S137.

SCALZO, J., POLITI, A., PELLEGRINI, N., MEZZETI, B. and BATTINO, M. 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition. *2*, 207–213.

SELLAPPAN, S., AKOH, C.C. and KREWER, G. 2002. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. J. Agric. Food Chem. *50*, 2432–2438.

SINGLETON, V.L. and ROSSI, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic – phosphotungstic acid reagents. Am. J. Enol. Vitic. *16*, 144–158.

SIRIWOHARN, T. and WROLSTAD, R.E. 2004. Characterization of phenolics in Marion and Evergreen blackberries. J. Food Sci. *69*, 233–240.

SIRIWOHARN, T., WROLSTAD, R.E., FINN, C.E. and PEREIRA, C.B. 2004. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. J. Agric. Food Chem. *52*, 8021–8030.

STURM, K., KORON, D. and STAMPAR, F. 2003. The composition of fruit of different strawberry varieties depending on maturity stage. Food Chem. *83*, 417–422.

SUN, J., CHU, Y.F., WU, X.Z. and LIU, R.H. 2002. Antioxidant and antiproliferative activities of common fruits. J. Agric. Food Chem. *50*, 7449–7454.

TULIPANI, S., MEZZETI, B., CAPOCASA, F., BOMPADRE, S., BEEKWILDER, J., RIC DE VOS, H.C., CAPANOGLU, E., BOVY, A. and BATTINO, M. 2008. Antioxidants, phenolic compounds, and nutritional quality of different strawberry genotypes. J. Agric. Food Chem. *56*, 696–704.

VANGDAL, E. and SLIMESTAD, R. 2006. Methods to determine antioxidative capacity in fruit. J. Fruit Ornam. Res. *14*(Suppl. 2), 123–131.

WANG, S.Y. and JIAO, H. 2000. Scavenging capacity of berry crop on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. J. Agric. Food Chem. *48*, 5677–5684.

WANG, S.Y. and LIN, H.S. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem. *48*, 140–146.

WANG, S.Y. and ZHENG, W. 2001. Effect of plant growth temperature on antioxidant capacity in strawberry. J. Agric. Food Chem. *49*, 4977–4982.

ZADERNOWSKI, R., NACZK, M. and NESTEROWICZ, J. 2005. Phenolic acid profiles in some small berries. J. Agric. Food Chem. *53*, 2118–2124.