Turk J Biol
36 (2012) 738-745

doi:10.3906/biy-1206-39

A comparison of major taste- and health-related compounds of *Vaccinium* berries

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Received: 18.06.2012 ● Accepted: 02.08.2012

Abstract: The chemical fruit composition, phenolic profile, and corresponding total antioxidant activity of bilberry (*Vaccinium myrtillus*) and 2 commercial blueberry cultivars (*Vaccinium corymbosum*) were analyzed. Results from this study showed that cultivar Berkeley yielded the highest glucose and fructose contents (70.8 and 88.8 mg/g FW, respectively), and the sweetness index expressed a similar trend, achieving the highest value for cultivar Berkeley (279.2). Citric acid was the major organic acid in the berries tested. *V. myrtillus* yielded a total organic acids value 2-fold higher (0.35 mg/g FW) as well as the highest vitamin C content (25.8 mg/100 g FW). Among the flavonols measured, myricetin was most abundant in *V. myrtillus* (10.7 µg/g FW), whereas the highest amounts of kaempferol and quercetin were detected in the blueberry cultivar Bluecrop (4.75 and 9.11 µg/g FW, respectively). Despite the challenge of characterizing phenolic acids due to the complexity and natural variation in fruit composition, this study confirms that cultivars of *V. corymbosum* are rich sources of chlorogenic acid, particularly cultivar Bluecrop (71.2 µg/g FW). Nevertheless, total phenolics were higher in *V. myrtillus* than in the cultivated blueberries, and consequently the highest level of total antioxidant capacity was recorded in wild bilberry (6.16 mg asc/g FW). This semicomprehensive study characterizes the fruit quality attributes and illustrates differences in the content of taste- and health-related compounds present in these *Vaccinium* berries.

Key words: Bilberry, highbush blueberry cultivars, sugars, organic acids, phenolics, antioxidant capacity

Introduction

Epidemiological studies suggest that consumption of fruit and vegetables contributes to a reduced risk of certain types of human cancer and cardiovascular diseases (1). Among fruits, berries are popular because of their good taste and well-known nutritional value. Indeed, *Vaccinium* berries such as bilberries (*Vaccinium myrtillus* L.) and blueberries (*Vaccinium corymbosum* L.) contain high amounts of sugars and acids as well as phenolic compounds that display potential health-promoting effects (2,3). Since fruit taste depends not only on total sugar and organic acid contents but also on the type and quantity of individual compounds, the composition of compounds may reflect changes in fruit quality (4). There is a lack of information that addresses how genotype affects the concentration of sugars and acids in the fruit, and these levels can act as an index of consumer acceptability (5). Nevertheless, among the factors affecting fruit quality, concentrations of soluble sugars are most commonly measured in

relation to the organoleptic factors of sweetness, acidity, astringency, and overall flavor perception (6). Sugars in blueberry fruit are mainly mono- and disaccharides (glucose, fructose, and sucrose), and the relative proportions of these individual sugars are important for perception of sweetness (7).

An important question that arises from the preliminary screening of *Vaccinium* berries relates to the polyphenolic profiles of the species, specifically, which components within these profiles contribute the most to differences in bioactive potential. These compounds are mainly represented by flavonoids, phenolic acids, and tannins, which are known as natural antioxidants (8–10). Flavonoids are polyphenolic phytochemicals that constitute a large group of secondary plant metabolites. Among them, flavonols such as quercetin, kaempferol, and myricetin and their derivatives (primarily glycosides) are considered the dominant flavonoids in bilberries, cranberries, and lingonberries (11). Additionally, these berries are known for a phenolic acid content that constitutes about one-third of the dietary phenols and may provide health benefits as a dietary antioxidant (12). According to Zheng and Wang (13), chlorogenic acid is the predominant phenolic acid in blueberries and accounts for 20.9% of total antioxidant activity. Ellagic acid has also been detected in some blueberry cultivars and could be present as ellagitannins, free ellagic acid, and ellagic acid glycosides (14). Interest in ellagic acid has increased during the past decade due to its possible antimutagenic, anticarcinogenic, and antioxidative effects (15).

Taking into account the importance of fruit quality attributes including the antioxidant capacity of flavonoids and phenolic acids, the purpose of this study was to determine and quantify important taste- and health-related compounds in *Vaccinium* berries. The variability in chemical fruit composition, specifically for sugars, organic acids, and identified phenolic compounds, and the correlation between total phenolic content and antioxidant capacity, highlight genetic differences among cultivated highbush blueberries and wild bilberry (*V. myrtillus*). It may provide a better understanding of the role of the wild species as an important native genetic source for breeding cultivars with higher nutritional value. This research also supports an extended view of consumer-oriented quality in which health-promoting bioactive compounds are desired quality attributes. This view is gaining importance among blueberry growers and other actors in the food distribution chain.

Materials and methods

Fruit sample handling and extract preparation

Wild bilberries (*Vaccinium myrtillus* L.) were harvested from native populations in Western Serbia (municipality of Dragačevo) and compared to the berries of 2 highbush blueberry cultivars of *Vaccinium corymbosum* L. (Berkeley and Bluecrop) harvested at a commercial plantation located in the same region as the studied wild species. Fruit samples were collected during a 3 year period from 2008 to 2010, and special care was taken to pick berries of the appropriate maturity stage from all sections of the bush.

Samples consisting of 50 fruits 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 3000 × *g* for 15 min. The methanol supernatant was divided into aliquots and frozen at −80 °C until analysis. Triplicate extractions were prepared for each berry 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, USA).

Determination of sugars and sweetness index

Separations were performed on a Waters Breeze chromatographic system (Waters, Milford, MA, USA) containing a 1525 binary pump system, thermostated column compartment, and 2465 Waters electrochemical detector equipped with a gold working electrode and hydrogen referent electrode. The separation of sugars was by CarboPac PA1 (Dionex, Sunnyvale, CA, USA) 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 a constant temperature of 30 °C. Signals were detected in pulse mode with the following waveform: E_1 = +0.1 V for 280 ms, E_2 = +0.75 V for 150 ms, and E_3 = −0.85 V for 150 ms and within 80 ms of integration time. Filter timescale was 0.2 s, and the range was 200–500 nA for the full mV scale. Data acquisition and evaluation were carried out by Waters Empower 2 software.

The sweetness index was calculated by multiplying the sweetness coefficient of each individual sugar (glucose = 1, fructose = 2.3, and sucrose = 1.35), as described by Keutgen and Pawelzik (5).

Determination of organic acids content

Separation of organic acids was performed by a Hewlett Packard HP1100 series chromatograph (Palo Alto, CA, USA) composed of inline degasser, autosampler, thermostated compartment, and HP 1100 photo diode array detector adjusted to 210 nm, with a reference signal at 600 nm. An anion exchange column (Aminex HPX-87H, Bio-Rad Lab, CA, USA) 300 × 7.8 mm was used with 5 mM H_2SO_4 as a mobile phase. The elution used was isocratic with a flow rate of 0.6 mL/min at 30 °C. Data acquisition and evaluation were carried out by Agilent Chemstation software.

Determination of vitamin C content

Ascorbic acid was measured by reflectometer (Merck RQflex, Darmstadt, Germany) as described by Pantelidis et al. (16). Results were expressed as milligrams of ascorbic acid per 100 grams of fresh weight (mg/100 g FW).

Determination of flavonols and phenolic acids content

Quantification of individual phenolic compounds was done by reversed phase HPLC analysis. Samples were injected in a Waters HPLC system consisting of 1525 binary pumps, thermostat, and 717+ autosampler connected to a Waters 2996 diode array detector (Waters, Milford, MA, USA). 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) with a 5 μm particle diameter (Waters, Milford, MA, USA) connected to the appropriate guard column. With the following gradient profile 2 mobile phases, A (0.1% phosphoric acid) and B (acetonitrile), were used at a flow of 1 mL/min: first 20 min from 10%–22% B, next 20 min of linear rise up to 40% B followed by 5 min reverse to 10% B, and an additional 7.5 min of equilibration time. Data acquisition and spectral evaluation for peak confirmation were done by Waters Empower 2 Software (Waters, Milford, MA, USA).

Determination of total phenolics (TPH)

The method employed was based on Folin–Ciocalteu phenol reagent and spectrophotometric determination (17). Results were expressed as milligrams of gallic acid equivalent per gram of fresh weight (mg GAE/g FW).

Determination of the total antioxidant capacity (TAC)

TAC was measured by ABTS method according to Arnao et al. (18). 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 25 °C. 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 gram of fresh weight (mg asc/g FW).

Statistical analysis

Statistical analysis was performed using Statistica 6.0 for Windows (StatSoft Inc., Tulsa, OK, USA). Data from the 3-year investigation were calculated by MANOVA for mean comparison, and intergenotype significance of difference was calculated by LSD test. Data are reported as means ± standard error of the mean. Correlations between TPH and TAC were calculated using Pearson's test. Differences at $P \leq 0.05$ were considered statistically significant.

Results and discussion

Sugars content and sweetness index

Sugars content and sweetness index in wild bilberry (*V. myrtillus*) and highbush blueberry cultivars (Berkeley and Bluecrop) are presented in Table 1. The main individual sugar was fructose, followed by glucose in all berries tested. Sucrose was present in much lower quantities due to the fact that it may be converted to inverted forms during the ripening process. The highest average amounts of glucose and fructose were detected in cultivar Berkeley (70.8 and 88.8 mg/g FW, respectively); however, no significant differences were observed in fructose content among berries tested. Total sugars content varied from 153.2 mg/g FW (*V. myrtillus*) to 162.7 mg/g FW (Berkeley).

The sweetness index showed a trend similar to differences in sugar distribution between wild bilberry and highbush blueberry cultivars, achieving the highest value for cultivar Berkeley (279.2 mg/g FW). *V. myrtillus* expressed somewhat lower relative units in the sweetness index (274.9 mg/g FW) due to the lower amounts of individual sugars in their fruits.

Organic acids and vitamin C content

Taste of berries is not only influenced by sugars. Acids within the fruit are also important contributors to its taste and flavor (19). In the present study citric and malic acid, 2 major organic acids, were found in the wild bilberry and the cultivated blueberries (Table 2). The main organic acid was citric acid, whereas quantities of malic acid that were twice as low were observed in all berries tested. The total organic acids content was identical in the blueberry cultivars studied (0.16 mg/g FW), and the value was 2-fold higher in *V. myrtillus* (0.35 mg/g FW). Variations in organic acid metabolism have been reported for many fruits (20), and several genetic studies have shown that the accumulation of organic acids (malic and citric) is controlled by genes that differ not only among species but also among cultivars (21).

In this study the wild species *V. myrtillus* had the highest amount of vitamin C (25.8 mg/100 g FW). Significantly lower and similar amounts of vitamin C were observed in highbush blueberry cultivars Berkeley and Bluecrop (9.69 and 8.08 mg/100 g FW, respectively). This is in line with the findings of Häkkinen et al. (11). With an average content ranging from 9 to 16 mg/100 g FW, the vitamin C level is not high enough to classify blueberries as a rich source of vitamin C among fruits (22). According to these authors, the share of vitamin C in TAC measured by the ORAC method did not exceed 3%. This confirms that the TAC of bilberry and cultivated blueberries originates mainly from other phytochemicals contained in the fruit, not from ascorbic acid.

Flavonols and phenolic acids content

Blueberry fruits are an important source of health-related compounds. Among these, the present work gives special attention to flavonols and phenolic acids concentrations (Table 3). Since flavonols belonging to the flavonoid group are considered most relevant to human health (23), 3 flavonols (kaempferol, myricetin, and quercetin) were detected in bilberry and blueberry cultivars. The obtained

Table 1. Sugars content and sweetness index in bilberry (*V. myrtillus* L.) and highbush blueberry cultivars (*V. corymbosum* L.).

Species/cultivar	Sugars (mg/g FW)			Total sugars (mg/g FW)	Sweetness index
	glucose	fructose	sucrose		
V. myrtillus L. bilberry	57.8 ± 4.77^{c}	87.1 ± 7.08^{a}	8.3 ± 2.03^{a}	153.2 ± 13.5^{b}	274.9^{a}
V. corymbosum L. Berkeley	70.8 ± 3.28^{a}	88.8 ± 3.84^{a}	3.1 ± 1.20^{b}	162.7 ± 7.2^{a}	279.2^{a}
V. corymbosum L. Bluecrop	64.4 ± 1.86^{b}	84.4 ± 2.82^{a}	7.6 ± 0.73^{a}	156.4 ± 4.6^{b}	268.8^{b}

Mean of 3-year values with 3 replications in every year. Values are expressed as mean ± standard error. For each parameter different letters indicate significant differences at $P \leq 0.05$ between bilberry and cultivars. FW = fresh weight. Sweetness index = (glucose × 1) + (fructose × 2.3) + (sucrose × 1.35).

Table 2. Organic acids and vitamin C contents in bilberry (*V. myrtillus* L.) and highbush blueberry cultivars (*V. corymbosum* L.).

Species/cultivar	Organic acids (mg/g FW)			Vitamin C (mg/100 g FW)
	citric	malic	total acids	
V. myrtillus L. bilberry	0.23 ± 0.02[a]	0.12 ± 0.01[a]	0.35 ± 0.03[a]	25.8 ± 1.85[a]
V. corymbosum L. Berkeley	0.10 ± 0.01[b]	0.06 ± 0.02[b]	0.16 ± 0.02[b]	9.69 ± 0.24[b]
V. corymbosum L. Bluecrop	0.11 ± 0.01[b]	0.05 ± 0.00[b]	0.16 ± 0.03[b]	8.08 ± 0.10[b]

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

Table 3. Flavonols and phenolic acids contents in bilberry (*V. myrtillus* L.) and highbush blueberry cultivars (*V. corymbosum* L.).

Species/cultivar	Flavonols (µg/g FW)			Phenolic acids (µg/g FW)	
	kaempferol	myricetin	quercetin	ellagic acid	chlorogenic acid
V. myrtillus L. bilberry	3.04 ± 0.42[b]	10.7 ± 2.13[a]	7.10 ± 0.40[b]	8.02 ± 0.62[b]	19.9 ± 1.68[c]
V. corymbosum L. Berkeley	1.47 ± 0.16[c]	1.72 ± 0.33[b]	7.73 ± 0.52[b]	6.10 ± 0.41[c]	41.0 ± 3.25[b]
V. corymbosum L. Bluecrop	4.75 ± 0.79[a]	1.76 ± 0.42[b]	9.11 ± 0.62[a]	19.1 ± 2.94[a]	71.2 ± 7.46[a]

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

results indicated that myricetin was the predominant flavonol in *V. myrtillus* (10.7 µg/g FW), whereas the blueberry cultivar Bluecrop was characterized by the highest amounts of kaempferol and quercetin (4.75 and 9.11 µg/g FW, respectively). On the other hand, Häkkinen et al. (11) did not detect kaempferol in any *Vaccinium* berries. This may be explained by the influence of different abiotic environmental factors (temperature, water deficiency, irrigation, and nutrient stress), which lead to the appearance of various forms of kaempferol glycosides. However, higher quercetin and myricetin concentrations were also found in wild berries of the family *Ericaceae*, which is in accordance with the results obtained in this study. Cho et al. (24) reported that the flavonols in blueberries were predominately quercetin glycosides, which accounted for 75% of total flavonols in the genotypes tested.

The most abundant non-flavonoid blueberry phenolics are the hydroxycinnamic acid esters, especially chlorogenic acid (23). Chlorogenic acid contributes to the tart taste of fruit and fruit products, and in the presence of polyphenol oxidase it can be easily oxidized and further transformed into brown-colored compounds (2,25). The amounts of chlorogenic acid detected in the present study were significantly different between *V. myrtillus* and the commercial blueberry cultivars. *V. myrtillus* had the lowest chlorogenic acid content value (19.9 µg/g FW), whereas higher quantities ranging from 41.0 µg/g FW (Berkeley) to 71.2 µg/g FW (Bluecrop) were detected in the blueberry cultivars. Taruscio et al. (26) reported that domesticated half-highbush and highbush blueberry species contained the highest levels of chlorogenic acid, exhibiting on average 1414 and 1261 µg/g FW, respectively. The presence of chlorogenic acid in highbush blueberries was also confirmed by Kalt et al. (3). In contrast to our results, Sellappan et al. (14) did not detected chlorogenic acid in southern highbush blueberries; however, 5 other phenolic acids

were identified: gallic, caffeic, ferulic, *p*-coumaric, and ellagic. This may be partially explained by varietal differences or the different extraction procedures employed. In this study free ellagic acid levels observed in bilberry and cultivar Berkeley are also quite low (8.02 and 6.10 μg/g FW, respectively), and their detection is probably the result of acid hydrolysis products from ellagitannin breakdown (27). Moreover, Šavikin et al. (28) reported that free ellagic acid was not present in fresh and frozen bilberries or bilberry jam. In general, its content in berries varies among species and cultivars, but it can also be affected by growth conditions including environmental factors and cultivation techniques (29,30). Our results clearly showed that flavonols represent a major class of total phenolics in *Vaccinium* berries, but the proportion of chlorogenic acid was also much higher than previously reported (3,13,14).

Total phenolics and antioxidant capacity

Since the antioxidant capacity of individual phenolic compounds cannot always be evaluated due to potential interaction among compounds, determination of TAC facilitates a more realistic evaluation of the protective effects of the analyzed fruit (31). Total phenolics content is known to influence antioxidant and other health-related bioactivities; therefore, this study aimed to quantify the TPH content in wild bilberry and 2 commercial blueberry cultivars (Table 4). The very high TPH content of *V. myrtillus* (3.87 mg GAE/g FW), 2-fold greater than blueberry cultivars, shows its potential as a source of natural antioxidants. Reported TPH contents for *V. myrtillus* differ markedly (2,24,28,32); however, the TPH values determined in the present study were lower than those reported by Šavikin et al. (28) and Li et al. (33). Significant differences in TPH were also observed between the 2 commercial blueberry cultivars tested; values ranged from 1.50 mg GAE/g FW (Berkeley) to 1.99 mg GAE/g FW (Bluecrop). These amounts are slightly lower than those previously reported for cultivar Bluecrop (24,34). Differences between the present results and those reported in the literature may be associated with diverse cultivation and climate conditions or the use of different extraction solvents during sample preparation. Castrejón et al. (32) examined phenolic profiles and the quantitative composition of blueberries as well as corresponding antioxidant activity during fruit maturation and ripening and observed that TPH content and TAC tended to decrease during ripening.

The TAC results presented here showed similar levels for the 2 highbush blueberry cultivars tested (Berkeley and Bluecrop), probably reflecting similar total phenolic contents. The wild bilberry (*V. myrtillus*) exhibited the highest TAC level with an average value of 6.16 mg asc/g FW, although our findings on phenolic acids composition indicated that chlorogenic and ellagic acids were present at lower levels in bilberry (*V. myrtillus*) than in cultivated blueberries. However, *V. myrtillus* is a richer source of total anthocyanins and cyanidin-based anthocyanins than *V. corymbosum*, and their extracts are the most potent of the antioxidant solutions tested (35). Such results confirm the need to evaluate the diversity of native populations of *Vaccinium* species, and, based

Table 4. Pearson's correlation coefficients (r_{xy}) between total phenolics and total antioxidant capacity in bilberry (*V. myrtillus* L.) and highbush blueberry cultivars (*V. corymbosum* L.).

Species/cultivar	Total phenolics (mg GA/g FW)	Antioxidant capacity (mg asc/g FW)	Correlation coefficients (r_{xy})
V. myrtillus L. bilberry	3.87 ± 0.55[a]	6.16 ± 0.98[a]	0.95**
V. corymbosum L. Berkeley	1.50 ± 0.20[c]	2.51 ± 0.52[b]	0.95**
V. corymbosum L. Bluecrop	1.99 ± 0.30[b]	2.45 ± 0.43[b]	0.89**

Data are shown as mean ± standard error. For total phenolics and total antioxidant capacity different letters indicate significant differences at $P \leq 0.05$ between bilberry and cultivars. FW = fresh weight; r = coefficient of correlation; **= significant at $P \leq 0.01$.

on this, well-focused breeding programs can create new cultivars specifically selected for improved antioxidant potential.

As noted before (29,36,37), antioxidant capacity correlates well with total phenolic content in blueberries, confirming the possibility of using a total phenolics parameter as an indicator of antioxidant capacity. In this study, TAC was closely correlated with TPH in all berries tested (Table 4). Correlation coefficients were high and statistically significant at $P \leq 0.01$, ranging from $r = 0.89$ (Bluecrop) to $r = 0.95$ (*V. myrtillus* and Berkeley). Obtained data confirm the previously established finding for 16 highbush and interspecific hybrid cultivars grown at different locations in the USA (26). Although Prior et al. (38) showed variations in antioxidant capacity among 19 named cultivars representing several species, no significant differences were observed in the assessment of antioxidant capacity of the 2 commercial highbush blueberry cultivars involved in this study.

This semicomprehensive analysis characterizes the phytochemical profiles and illustrates differences in the content of taste- and health-related compounds present in these *Vaccinium* berries. In the case of sugar content, the 2 commercial blueberry cultivars of *V. corymbosum* investigated produced higher quantities than wild bilberry (*V. myrtillus*); the sweetness index was highest in cultivar Berkeley due to the higher amounts of individual sugars contained in the fruits. Conversely, *V. myrtillus* contained abundant quantities of total organic acids and vitamin C, an important attribute of nutritional fruit quality. The profile and content of phenolics present in these berries indicate that cultivar Bluecrop accumulated higher quantities of the most frequently measured individual phenolic compounds, e.g. flavonols (kaempferol and quercetin) and phenolic acids (chlorogenic and ellagic acids). These results are confusing as the highest TPH and TAC values were obtained in *V. myrtillus*, although myricetin was the only predominant flavonol observed. This would mean that phenolic compounds not included in this investigation, such as specific anthocyanin or flavonol derivatives, may also contribute to the TAC of *V. myrtillus*. In general, TPH values presented in the current study followed a pattern similar to TAC in all samples tested. Therefore, it is evident that TAC was strongly correlated to TPH levels and achieved the highest value in *V. myrtillus*. From a review of this study, it becomes apparent that bioactive characteristics of *Vaccinium* berries can be directly related to their specific phenolic profile. Furthermore, previous studies did not compare complete chemical fruit composition, including the important taste- and health-related compounds, in common commercial highbush blueberry cultivars and wild bilberry. This phytochemical profiling of *Vaccinium* berries represents an analysis of fruit quality components and, thus, may lead to a better understanding of their nutritional health benefits.

Acknowledgments

We are grateful for the financial support provided by the Serbian Ministry of Education and Science (projects 46008, 173040, and 173017).

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