

Evaluation of Phenolic Content and Antioxidant Capacity of Blueberry Cultivars (*Vaccinium corymbosum* L.) Grown in the Northwest Croatia

Verica Dragović-Uzelac^{1*}, Zvonimir Savić², Ana Brala¹, Branka Levaj¹,
Danijela Bursać Kovačević¹ and Ante Biško²

¹Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6,
HR-10000 Zagreb, Croatia

²Croatian Centre for Agriculture, Food and Rural Affairs, Jandrićeva 42, HR-10000 Zagreb, Croatia

Received: May 28, 2009

Accepted: January 27, 2010

Summary

The aim of this study is to evaluate the content of phenolics: total phenols (TPC), flavonoids (TF), anthocyanins (TA), flavan-3-ols (TF3ols), as well as total antioxidant capacity (TAC) and reducing power (RP) in four blueberry cultivars (*Vaccinium corymbosum* L.) introduced in the Northwest Croatian climate conditions. Phenolic compounds were measured by spectrophotometric methods, TAC was determined using DPPH and ABTS assays and RP by FRAP assay. All cultivars contained high mass fraction of TPC, TF, TA and lower mass fraction of TF3ols. Among the researched fruits, Sierra cultivar contained the highest amounts of all groups of phenolics, followed by Elliott>Bluecrop≥Duke. Significant differences were observed in phenolic mass fraction among different cultivars and growing seasons ($p<0.05$), and phenolic compounds were significantly higher in growing season 2006. Examined cultivars possess high antioxidant capacity and reducing power, and all phenolics were highly correlated with TAC and RP ($R=0.46$ to 0.99). The study indicated that growing and climate conditions in Northwest Croatia are convenient for introducing blueberry cultivars. Generally, blueberry fruits are a rich source of phenolics, which show evident antioxidant capacity.

Key words: blueberry, phenolics, antioxidant capacity, reducing power

Introduction

'Cultivated' highbush blueberries (*Vaccinium corymbosum* L.) are native to North America and have been commercially produced for many years, while recently the areas planted with blueberries in Europe have systematically been increasing. Since the plantations can be exploited for thirty years, the proper choice of cultivars is crucial. Highbush blueberry (*Vaccinium corymbosum* L.) breeders try to introduce new valuable cultivars and poorly chosen cultivars must be replaced with new, more valuable ones (1). Blueberries (*Vaccinium corymbosum* L.) are considered to be one of the richest sources of pheno-

lic compounds (2,3), and they contain significant levels of anthocyanins, flavonols, chlorogenic acid and procyanidins, which have high biological activity (4). The predominant flavonoids found in berries are anthocyanins and flavonols, which are almost exclusively present in glycosylated forms. Anthocyanins can also exist as diglycosides or as acylated forms of glycosides (5). There have been many attempts to determine the content and physiological activity of phenolic compounds in blueberries, due to the apparent relationship of phenolics in plant foods with the prevention of chronic diseases (3). Along with other antioxidant components, phenolics as natural secondary metabolites have been reported to be poten-

*Corresponding author; Phone: ++385 1 4605 128; Fax: ++385 1 4605 072; E-mail: vdragov@pbf.hr

tial components in reducing the number of cardiovascular disease events (6,7). The protective effects could be due to their properties as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers and/or metal ion chelators.

There are many factors that influence the phenolic content and antioxidant capacity of blueberries. Results obtained from several studies suggest that the composition and content of phenolic compounds in blueberries are influenced by the cultivar, the growing season and the growing location. Variation in total phenolics, anthocyanins, flavonoids and fruit mass among genotypes was much greater than that observed between growing seasons, indicating that genetics plays a more important role than growing season in influencing phenolic content in blueberries (8). It has been reported that a combined effect of growing season and genotype on anthocyanins and fruit mass demonstrates that environmental growth conditions can have a significant impact on the levels of phenolics in blueberries. In addition, genotypes with smaller berries had higher levels of total phenolics, anthocyanins and flavonoids than large-berried genotypes. Factors that have an impact on the antioxidant capacity include the total anthocyanin content, total phenolic content, maturity, and the postharvest storage conditions (2,9–11). Prior *et al.* (2) reported that correlation coefficient was much higher between total antioxidant capacity and the total phenolics compared to total antioxidant capacity and anthocyanins. Connor *et al.* (11) reported that antioxidant capacity, total phenolic content, and anthocyanin content were strongly correlated with each other ($R=0.87-0.99$, $p<0.01$). Correlation coefficients between anthocyanins or total phenols and FRAP value are significant across the *Vaccinium*, *Rubus* and *Ribes* sample sets and their subsets. Survey of small fruit germplasm for anthocyanins, total phenols, and antioxidant capacity confirms each as an excellent source of dietary phytochemicals (12). Furthermore, it was observed that late harvests of *V. ashei* Reade cultivars Tifblue and Brightwell demonstrated higher antioxidant activity than early harvests, as determined by their oxygen radical absorbance capacity (ORAC) (2,13). Several studies have demonstrated that the phenolic content and antioxidant capacity of blueberry (*Vaccinium L.*) fruit can be influenced by genetic differences (2,14).

There are no studies on compositional and physical properties of *Vaccinium* cultivars growing in Croatian climate conditions. Therefore, in this study four introduced blueberry cultivars, Duke (early season ripening), Sierra and Bluecrop (mid-season ripening) and Elliott (late season ripening) were evaluated under Northwest Croatian growth conditions. The objective of the present study is to evaluate the content of phenolics (total phenols, flavonoids, flavan-3-ols and anthocyanins) as quality markers as well as antioxidant capacity and reducing power in the mentioned blueberries, depending on the cultivar and growing season (2006 and 2007).

Materials and Methods

Plant material and its preparation

At the Hellea Ltd. (Zagreb, Croatia), a highbush blueberry production project started in 2004 in the vicinity of

Donja Bistra. The experiment was conducted at a commercial highbush blueberry field (45° 54' north latitude, 15° 50' east longitude) from 2006 to 2007. The field consisted of four-year-old homogenous plants. Four plants of cultivars Duke (early season ripening), Sierra and Bluecrop (both mid-season ripening) and Elliott (late season ripening) were planted per block. The plants were grown in a substrate consisting of peat, milled conifer chips and sawdust in equal proportions. After planting the substrate, acidity was adjusted to pH=4.2–4.5. Planting distance in the row was 1.5 m and the space between rows was 3.0 m. In addition to the irrigation system put in place, the trial plantation was equipped with the crop protection net. The experimental design used in this experiment consisted of a randomized complete block with four cultivars and three replications. The fertilizer used consisted of N:P:K=14:9:15 with trace elements and micronutrients applied at 60 kg/ha of N.

Fruit samples were harvested during the 2006 and 2007 growing seasons. The criteria for deciding on the appropriate fruit samples were: colour, percentage of berries on the bush at the particular maturity level, quality requirements (intact, healthy, clean, and free of abnormal external moisture) and the location of the fruit on the bush.

Maturity stage was decided on the basis of full blue colouration. Picking occurred when 40–70 % of fruit on the bushes were ripe. Overripe and damaged berries were removed from the sample. Berries were collected from all parts of the bush.

In 2006 fruit samples were harvested from cultivar Duke on July 8, Sierra July 19, Bluecrop July 19 and Elliott August 10. In 2007 fruit samples were harvested from cultivar Duke on June 18, Sierra June 26, Bluecrop July 3 and Elliott July 19. Three repetitions were sampled per cultivar ($m=150$ g/cultivar/repetition). After harvesting at the appropriate maturity, the samples of each cultivar were frozen using liquid nitrogen, packed in polyethylene bags and stored at -20 °C (for about 7 days). Before analysis a portion of fresh fruits was partially defrosted and homogenized in a house blender (Zepter, Mixy International).

Extraction of phenolics

Phenolics were extracted from partially defrosted blueberry fruits. Exactly 5 g of samples were weighed out and extracted using 20 mL of 80 % (by volume) aqueous ethanol. The mixture was extracted for 20 min in inert atmosphere (N_2), filtered through Whatman No. 40 filter paper (Whatman International Ltd, Kent, UK) using a Büchner funnel. Extraction of the residue was repeated using the same conditions. The filtrates were combined and adjusted to 50 mL in a volumetric flask with 80 % aqueous ethanol. The obtained extract was used for determination of total phenols (TPC), total flavonoids (TF), total flavan-3-ols (TF3ols), as well as for antioxidant capacity assay by using ABTS and DPPH methods, and reducing power assay by using FRAP method.

Determination of total phenolics (TPC)

For the determination of total phenols (TPC), the adjusted method (15,16) with Folin-Ciocalteu reagent was used. The content of TPC was measured as follows: 0.5 mL of diluted extracts or standard solutions of gallic acid

(20–500 mg/L) were added to a 50-mL volumetric flask containing 30 mL of double distilled water (ddH₂O), then 2.5 mL of Folin-Ciocalteu reagent were added to the mixture and shaken. After 5 min, 7.5 mL of 7 % Na₂CO₃ solution (*m/V*) were added with mixing and the solution was immediately filled up to 50 mL with ddH₂O. After incubation at room temperature for 2 h, the absorbance of the solution was measured by the spectrophotometer Unicam Helios β (Spectronic Unicam, Cambridge, UK) at 765 nm. The results were calculated according to the calibration curve for gallic acid ($y=0.0009x$, y =absorbance at 765 nm, x =concentration of gallic acid in mg/L, $R^2=0.9986$). The content of TPC was expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh mass (fm) of edible part of fruits.

Determination of total flavonoids (TF)

The total flavonoid (TF) assay was done as previously described by Zhuang *et al.* (17) with minor modifications. Modification was adjusted in extraction procedure, and the extract of total phenolics was used for TF determination as described above. A volume of 1 mL of diluted extracts or standard solution of rutin (50–500 mg/L) was placed in a 10-mL volumetric flask, then 4 mL of ddH₂O, and after 5 min 300 μL of NaNO₂ (1:20) and 3 mL of AlCl₃ (1:10) were added. The mixture was shaken and 6 min later 2 mL of 1 M solution of NaOH were added, again well shaken and centrifuged for 5 min at 5000 rpm. The supernatant was decanted and the absorbance was measured at 510 nm against the blank. The results were calculated according to the calibration curve for rutin ($y=0.00029x$, y =absorbance at 510 nm, x =concentration of rutin in mg/L, $R^2=0.9994$). The content of TF was expressed as mg of rutin equivalents (RE) per 100 g of fm of edible part of fruits.

Determination of total flavan-3-ols (TF3ols)

The vanillin assay was done as previously described (15,18,19) in the same extracts as TPC and TF. The content of flavan-3-ols was measured as follows: 5 mL of diluted extracts or standard solution of (+)-catechin (2–20 mg/L) were added to a 25-mL volumetric flask, then 10 mL of 11.5 M aqueous solution of HCl (by volume) and 5 mL of ethanol solution of vanillin reagent (1 %, *m/V*) were added and the mixture was adjusted to 25 mL with 96 % ethanol and shaken. The absorbance was measured at 500 nm after 20 min. The results were calculated according to the calibration curve for (+)-catechin ($y=0.0182x$, y =absorbance at 500 nm, x =concentration of (+)-catechin in mg/L, $R^2=0.9981$) and the results were expressed as mg of catechin equivalents (CE) per 100 g of fm of edible part of fruits.

Determination of total anthocyanins (TA)

The total anthocyanin (TA) content in the extract from selected fruits was determined using bisulphite bleaching method (20). Anthocyanins were extracted from 2 g of fresh samples of the fruits using 2 mL of 0.1 % HCl (by volume) in 96 % ethanol and 40 mL of 2 % aqueous HCl (by volume). The mixture was centrifuged at 5500 rpm for 10 min. The obtained supernatant was used for determination of TA. The content of TA was measured as follows: 10 mL of the extract were placed into two

test tubes, then 4 mL of 15 % sodium bisulphite were added to one test tube (A_2) and 4 mL of ddH₂O to the other (A_1). After a 15-minute incubation at room temperature, the absorbance of each mixture was measured at 520 nm. The total anthocyanins were calculated using the following equation:

$$A_0=615 \cdot (A_1-A_2) \quad /1/$$

where 615 represents molar absorptivity of cyanidin-3,5-diglucoside, A_1 the absorbance of mixture samples with ddH₂O and A_2 the absorbance of mixture samples with 15 % sodium bisulphite. Results were expressed as mg of cyanidin-3,5-diglucoside equivalents (CydGE) per kg of fm of edible part of fruits. Average results were obtained from three parallel determinations for all determined groups of phenolics (TPC, TF, TF3ols and TA).

Determination of total antioxidant capacity by ABTS and DPPH methods

ABTS [2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation assay

The radical scavenging capacity of fruit extracts was evaluated against ABTS generated by chemical method according to a previously reported one (21). The assay is based on the ability of antioxidant molecules to decolourise the radical cation 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonate) [ABTS^{•+}], a blue-green chromophore with characteristic absorption at 734 nm. The addition of antioxidants to the radical cation reduces it to ABTS. Briefly, ABTS radical cation (ABTS^{•+}) was produced by reacting 7 mmol/L of ABTS stock solution with 2.45 mM potassium persulphate (K₂S₂O₈) in the dark at room temperature for 12–16 h before use. The next day, ABTS^{•+} solution (1 %, by volume) was diluted with 96 % ethanol to an absorbance of (0.73±0.02) at 734 nm. After the addition of 0.16 mL of sample or 25–300 μM aqueous solution of Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) to 2 mL of diluted ABTS^{•+} solution, absorbance readings were taken after 1 min at 734 nm using the spectrophotometer Unicam Helios β (Spectronic Unicam, Cambridge, UK). Ethanol was used as a blank. The results were calculated according to the calibration curve for Trolox ($y=-0.00219x+0.678662$, y =absorbance at 734 nm, x =concentration of Trolox in μM, $R^2=0.9989$) and the ABTS values, derived from triplicate analyses, were expressed as mmol of Trolox equivalent (TE) per 100 g of fm of edible part of fruits.

DPPH (2,2-diphenyl-2-picrylhydrazyl) free radical assay

The free radical scavenging capacity of fruit extracts was determined according to the previously reported procedure using the stable DPPH radical (22). The method was based on the reduction of stable DPPH nitrogen radicals in the presence of antioxidants. An aliquot (2 mL) of diluted fruit extract or methanol solution of Trolox (10–30 mM) was mixed with 2 mL of methanol and 1 mL of 0.5 mM DPPH methanolic solution. The mixture was thoroughly vortexed, kept in the dark for 20 min, and after that the absorbance was measured at 517 nm against a blank of methanol without DPPH. The results were calculated according to the calibration curve for Trolox ($y=-0.00124x+1.203124$, y =absorbance at 517 nm,

x =concentration of Trolox in mM, $R^2=0.9991$). DPPH values, derived from triplicate analyses, were expressed as mmol of TE per 100 g of fm of edible part of fruits.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was conducted according to Benzie and Strain (23). This method is based on an increase of the absorbance at 593 nm due to the formation of tripyridyl-S-triazine complexes with Fe^{2+} [TPTZ- $Fe(II)$] in the presence of a reductive agent. The FRAP reagent was prepared from 2.5 mL of TPTZ solution (10 mmol/L) in hydrochloric acid (40 mmol/L) and 2.5 mL of $FeCl_3$ solution (20 mmol/L) mixed with 25 mL of acetate buffer (0.3 mol/L, pH=3.6). For the determination of the antioxidant capacity, the FRAP reagent (2.08 mL) was mixed with 240 μ L of water and 80 μ L of the appropriately diluted sample or standard solution of $FeSO_4 \cdot 7H_2O$ (25–750 mM). The mixture was allowed to stand for 5 min at room temperature before the absorption was measured at 593 nm (Unicam Helios β , Spectronic Unicam, Cambridge, UK). FRAP values, derived from triplicate analyses, were calculated according to the calibration curve for $FeSO_4 \cdot 7H_2O$ ($y=0.00053372x$, y =absorbance at 593 nm, x =concentration of $FeSO_4 \cdot 7H_2O$ in mM, $R^2=0.9979$), and they were expressed as mmol of Fe^{2+} equivalents (FE) per 100 g of fm of edible part of fruits.

Statistical analysis

The data were analyzed using Statistica v. 7 (Statsoft Inc, Tulsa, OK, USA). Analysis of variance (ANOVA) was used to compare all significant differences between blueberry cultivars and growing seasons. Differences were considered significant at $p<0.05$. Values were expressed as means ($N=3$) with standard deviations (S.D.). For comparison of the results of TPC, TF, TF3ols, TA and DPPH, ABTS or FRAP assays, the coefficients of correlation were determined for each combination.

Results and Discussion

Total phenols, flavonoids, flavan-3-ols and anthocyanins

The mass fractions of total phenols (TPC), total flavonoids (TF) and total flavan-3-ols (TF3ols) in four blueberry cultivars (Duke, Elliott, Sierra and Bluecrop) introduced in the Northwest Croatian climate conditions during

two growing seasons (2006 and 2007) are given in Table 1. TPC content in fruits harvested in 2006 determined with Folin-Ciocalteu assay ranged from 358.7 mg of gallic acid equivalents (GAE) per 100 g of fm in early season ripening cultivar Duke to 528.2 mg of GAE per 100 g of fm in mid-season ripening cultivar Sierra. In the same cultivars harvested in 2007, TPC content was lower and ranged from 279.3 to 331.3 mg of GAE per 100 g of fm. Comparing the results of our research with the results of other authors, the mass fraction of TPC in Duke cultivar grown in 2006 was in agreement with the findings of Zheng *et al.* (24), who found 313 mg of GAE per 100 g of fm of TPC in the same blueberry cultivar. Mass fractions of TPC in Bluecrop cv. (368.3 mg of GAE per 100 g of fm) were in the similar range like those by Prior *et al.* (2) and Connor *et al.* (11). Among the eight cultivars assayed, the values of total phenolics were found to be in the range from 181.1 to 390.5 mg per 100 g of fm expressed as gallic acid equivalents, whereas Bluecrop and Duke cultivars had 189.8 and 305.9 mg per 100 g of fm (2). According to Connor *et al.* (11), total phenolics ranged from 335 to 595 mg of chlorogenic acid per 100 g of fresh fruit, depending on the cultivar. Howard *et al.* (8) also found similar values for total phenols, and the evaluated cultivars grown in 2000 contained TPC in the range from low 202 mg per 100 g to very high 586 mg per 100 g of fresh mass, and reflecting a 2.9-fold difference among cultivars.

According to the results obtained in our research, it is obvious that in 2006 growing season, mid-season ripening cultivar Sierra and late season ripening cultivar Elliott contained higher amounts of total phenolics. In 2007 growing season, remarkable differences in TPC mass fraction among cultivars Duke, Elliott and Bluecrop were not observed, while in cv. Sierra, TPC was determined in remarkably high amount.

In total phenolic content, flavonoids were predominant, and their amounts varied from 269 mg RE per 100 g of fm in Duke cv. to 432.2 mg RE per 100 g of fm in Sierra cv. in 2006, whereas in 2007 their amounts ranged from 216.9 to 326.6 mg per 100 g of fm. It was calculated that percentages of TF in TPC varied between 75 and 82 % in blueberry fruits grown in 2006, and between 78 and 98 % in blueberry fruits grown in 2007. The obtained results suggest that flavonoids were the most abundant phenolics in blueberry fruits. Lata *et al.* (25) reported that the value of total flavonols (subgroup of

Table 1. Total phenols (TPC), flavonoids (TF) and flavan-3-ols (TF3ols) in blueberry cultivars in growing seasons 2006 and 2007

Cultivar	Growing season	TPC as $w(GAE)$ mg/100 g fm	TF as $w(RE)$ mg/100 g fm	TF3ols as $w(CE)$ mg/100 g fm
Duke	2006	358.70±18.61	268.97±21.53	95.75±10.08
	2007	279.27±7.72	216.87±13.55	72.18±11.05
Elliott	2006	476.85±22.83	376.68±16.83	101.89±10.05
	2007	264.06±20.75	255.33±13.64	73.05±9.80
Sierra	2006	528.15±22.37	432.18±13.94	133.28±6.49
	2007	331.34±7.72	326.58±12.55	105.11±15.64
Bluecrop	2006	368.33±22.73	278.73±17.36	72.99±0.57
	2007	291.56±4.71	236.87±13.30	64.27±12.22

Values are an average of 3 replications±S.D.

flavonoids) in TPC was about 25 % in fruits of Bluecrop cv, whereas in our Bluecrop cultivar the level of TF in TPC was 76 %. Comparing the level of TF in TPC between 2006 and 2007, it was observed that blueberries harvested in 2007 contained higher percentage of TF in TPC. Compared to the mass fractions of other phenolics present in blueberries, flavan-3-ols were not found in remarkable amounts, and the lowest amount was determined in Bluecrop cv. (73 mg CE per 100 g of fm). The mentioned value presented about 20 % of TF3ols in TPC. The highest amount of TF3ols was determined in Sierra cv. (133.3 mg CE per 100 g of fm) and TF3ols of about 25 % in TPC. In 2007, TF3ols in all cultivars were determined in lower amounts compared to the same cultivars grown in 2006, whereas TF3ols in TPC varied between 27 and 32 %. Generally, the amounts of all determined phenolics (TPC, TF and TF3ols) were higher in fruits grown in 2006, while in all cultivars grown in 2007, TF and TF3ols in TPC were present in a higher percentage. This could be due to climate conditions and the season of ripening. Comparing the growing seasons 2006 and 2007, environmental growth conditions showed differences. Meteorological and Hydrological Service data (Table 2) confirmed that in 2006 mean daily air temperature was 22.5 °C in July and 18 °C in August, and in 2007 it was 21.3 °C in June and 21.7 °C in July (26,27). Also, monthly sums of sunshine duration showed differences.

Cultivar with the lowest amount of phenolics was Duke, and this could be because it is an early season ripening cultivar, when lower temperature decreases biosynthesis of phenolics.

The amount of anthocyanins in four blueberry cultivars introduced in Northwest Croatian climate conditions is shown in Fig. 1. It is evident that Sierra cv. is the richest source of anthocyanins among the examined cultivars. The amount of anthocyanins in Sierra cv. varied between 197.3 (year 2007) and 244.6 mg per 100 g of fm (year 2006). In other cultivars, anthocyanins were present in reduced amounts as follows: Elliott>Duke>Bluecrop. The differences between anthocyanin concentrations were significant depending on the cultivar. Our results are similar in comparison with previous studies. The level of TA in TPC was 49 % for Bluecrop and 42 % for Duke cultivar. Lata *et al.* (25) reported that the value of total anthocyanins in TPC was 45 and 50 % depending on the growing season of fruits of Bluecrop cultivar. The anthocyanin content in blueberry in this study was comparable to the quantity reported by Prior *et al.* (2). According to the mentioned authors, total anthocyanins in Bluecrop cv. were 93.1 and for Duke cv. 127.4 mg per 100 g of fm.

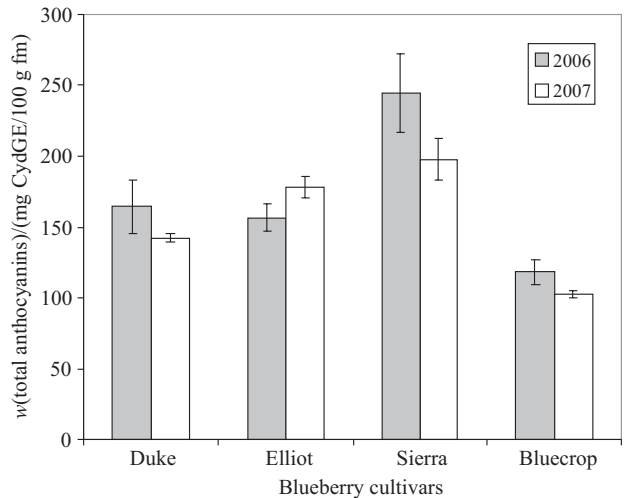


Fig 1. Total anthocyanins (mg CydGE/100 g fm) in blueberry cultivars in growing seasons 2006 and 2007

The research of Giovanelli and Buratti (28) showed that the total phenolic content of cultivated berries is quite homogenous ranging from 251 to 310 mg per 100 g of fm. Total anthocyanin mass fractions also lie in a narrow range (from 92 to 129 mg per 100 g of fm) and represent approx. 30–40 % of the total phenols in the four cultivated varieties. Connor *et al.* (11) found similar content of anthocyanins in Bluecrop (123 mg per 100 g of fm) and 191 mg per 100 g of fm in Elliott, while Howard *et al.* (8) obtained lower values for Bluecrop, *i.e.* comparing the growing seasons, Bluecrop had approx. 74 mg per 100 g of fm and total anthocyanin mass fraction in TPC was 29 %.

Generally, the blueberries cultivated in 2006 had significantly higher total content of all determined phenolics compared to those cultivated in 2007. The ANOVA (Table 3) showed significant differences among four blueberry cultivars ($p < 0.05$) as well as among two growing seasons ($p < 0.05$). Particularly significant differences were observed in the concentration of TF and TF3ols depending on both sources of variation (cultivars and growing season), whereas TPC concentration significantly depended on the growing season and TA depended on the cultivar.

Total antioxidant capacity (TAC) and reducing power (RP)

The total antioxidant capacity (DPPH and ABTS assays) and reducing power (FRAP assay) of four blueberry cultivars during growing seasons 2006 and 2007 are shown in Table 4. TAC and RP depend on mass frac-

Table 2. Mean monthly air temperatures and monthly sums of sunshine duration

Climate factor	Growing season	Month							
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Mean monthly air temperature/°C	2006	-2.1	0.8	4.9	11.7	15.4	19.7	22.5	18.0
	2007	6.0	6.3	8.0	13.1	17.3	21.3	21.7	19.9
Monthly sum of sunshine duration/h	2006	82.0	90.2	130.5	189.3	250.1	293.3	333.6	183.2
	2007	130.4	102.2	156.7	303.7	268.9	286.9	345.3	248.2

Data from Meteorological and Hydrological Service, Zagreb, Croatia (26,27)

Table 3. Analysis of variance (ANOVA) of the blueberry cultivars and growing season

Phenolics	Source of variation	F _{exp}	p-value
TPC	Cultivars	1.8632	0.3110
	Growing season	14.8354	0.0309*
TF	Cultivars	10.0847	0.0447*
	Growing season	16.7979	0.0263*
TF3ols	Cultivars	20.5384	0.0167*
	Growing season	22.7189	0.0175*
TA	Cultivars	9.9642	0.0455*
	Growing season	1.1341	0.3650

*Significant differences obtained at $p \leq 0.05$

tions of phenolics and are decreasing in the following order: Sierra>Elliott>Bluecrop>Duke (in most cases the values are equal). Total antioxidant capacity measured by DPPH method ranged from 5.6 to 7.6 mmol Trolox equivalent (TE) per 100 g of fm, whereas measured by ABTS method it ranged from 15.8 to 28.4 mmol TE per 100 g of fm. Giovanelli and Buratti (28) determined the antioxidant capacity by DPPH and FRAP methods. All values of antioxidant capacity were consistent. FRAP antioxidant capacity value for Bluecrop was 24.3 TE $\mu\text{mol/g}$. Linear correlations demonstrated that the antioxidant capacity values determined by DPPH and FRAP assays were slightly better correlated to total phenolics rather than to total anthocyanins.

The reducing power in this study was determined as the Fe^{3+} to Fe^{2+} transformation, and RP increased with increasing concentrations of phenolics in the blueberry extracts. The highest RP was observed in Sierra, followed by Elliott, Duke and Bluecrop. Correlation coefficients be-

tween TF or TF3ols and RP show that the mentioned group of phenolics contributed more to RP (Table 5). Reducing power is generally linked with the presence of reducing substances, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (29). The highest TAC and RP were observed in Sierra blueberry fruits, a cultivar which contained significantly higher amounts of all examined groups of phenolics. The TAC and RP of Duke and Bluecrop cultivars were lower than in other cultivars. The results of TAC and RP are in reasonable agreement with the content of phenolic compounds, which was confirmed by high correlation coefficients between phenolics and TAC, as well as phenolics and RP (Table 5). There have been many studies showing the correlation between phenolic compounds and antioxidant capacity. Šcibisz and Mitek (30) demonstrated that the antioxidant capacity was strongly correlated with the content of total anthocyanins and total phenolics. The correlation coefficient was higher between the antioxidant capacity and anthocyanins ($R=0.93$) compared to the antioxidant capacity and total phenolic content ($R=0.89$). These conclusions correspond to Giovanelli and Buratti's research (28). The data demonstrated that the antioxidant capacity values, determined by DPPH and FRAP, are better correlated with total phenolics ($R>0.985$) rather than with total anthocyanins ($R<0.87$). Koca and Karadeniz (29) observed a linear relationship between FRAP values and total phenolics for blueberries ($R=0.981$). The abundance of flavonoids (Table 1) could be the main contribution to the high TAC and RP (higher correlation coefficients) of blueberry fruits. Shahidi and Naczyk (31) reported that the differences in the antioxidant activities of fruits could be due to their different profiles of phenolic acids, flavonoid compounds and their derivatives. For instance, anti-

Table 4. Antioxidant capacity (DPPH, ABTS) and reducing power (FRAP) in blueberry cultivars in growing seasons 2006 and 2007

Cultivar	Growing season	$c(\text{DPPH})$	$c(\text{ABTS})$	$c(\text{FRAP})$
		mmol TE/100 g fm	mmol TE/100 g fm	mmol Fe^{2+} /100 g fm
Duke	2006	6.13±0.56	15.73±2.61	50.87±2.89
	2007	5.75±1.56	16.44±1.73	39.03±1.57
Elliott	2006	6.56±0.38	18.50±4.33	62.20±4.20
	2007	5.63±1.31	16.02±1.11	47.68±2.19
Sierra	2006	7.60±0.17	28.37±1.76	90.07±11.39
	2007	6.94±0.25	26.68±1.04	68.66±3.31
Bluecrop	2006	6.13±0.35	16.13±0.71	48.33±1.86
	2007	5.99±1.13	15.85±2.06	37.13±1.74

Values are an average of 3 replication±S.D.

Table 5. Correlation coefficients between phenolics and antioxidant capacity (DPPH, ABTS) or reducing power (FRAP)

Phenolics	Growing season 2006			Growing season 2007		
	DPPH	ABTS	FRAP	DPPH	ABTS	FRAP
TPC	0.84	0.78	0.85	0.97	0.84	0.57
TF	0.87	0.81	0.87	0.55	0.87	0.95
TF3ols	0.86	0.82	0.89	0.61	0.96	0.95
TA	0.87	0.85	0.88	1.00	0.46	0.73

oxidant activities of flavonoids are linked with the number of hydroxyl groups in their molecules. Generally, blueberry cultivars introduced in the Northwest Croatian climate conditions are an excellent source of dietary antioxidants and different groups of phenolics.

Conclusions

Generally, growing and climate conditions in Northwest Croatia are convenient for introducing blueberry cultivars (Duke, Elliott, Bluecrop and Sierra). This was proved with good quality of blueberry fruits, which contained high mass fractions of different groups of phenolics (particularly important flavonoids, especially anthocyanins) during two consecutive growing seasons. The richest source of TPC, TF, TF3ols and TA among cultivars was Sierra, followed by Elliott, Bluecrop and Duke. Antioxidant capacity and reducing power reached the highest levels in Sierra and Elliott blueberry fruits. Nutritionally, the mentioned cultivars possess a higher functional benefit than other evaluated cultivars. Some blueberry cultivars introduced in the Northwest Croatian climate conditions contained mass fractions of phenolics similar to the same cultivars grown in other regions. They possess high concentration of phenolics, antioxidant capacity and reducing power. On the basis of the obtained results and considering the mass fraction of anthocyanins and flavonoids, it could be concluded that the analyzed cultivars could be suitable for cultivation in Northwest Croatia. However, future studies should include additional analyses to obtain a complete evaluation of the quality of fruits.

Acknowledgements

The authors are grateful to the Institute for Fruit Growing (Zagreb, Croatia) for financial support and to Hellea company (Zagreb, Croatia) for providing the berry samples.

References

1. K. Smolarz, Evaluation of four blueberry cultivar growing in Central Poland, *Acta Hort.* 715 (2006) 81–84.
2. R.L. Prior, G. Cao, A. Martin, E. Sofic, J. McEwen, C. O'Brien, N. Lischner, M. Ehlenfeldt, W. Kalt, G. Krewer, C.M. Mainland, Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of *Vaccinium* species, *J. Agric. Food Chem.* 46 (1998) 2686–2693.
3. A.D.R. Castrejón, I. Eichholz, S. Rohn, L.W. Kroh, S. Huyskens-Keil, Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening, *Food Chem.* 109 (2008) 564–572.
4. M.J. Cho, L.R. Howard, R.L. Prior, J.R. Clark, Flavonol glycosides and antioxidant capacity of various blackberry and blueberry genotypes determined by high-performance liquid chromatography/mass spectrometry, *J. Sci. Food Agric.* 85 (2005) 2149–2158.
5. M.J. Cho, L.R. Howard, R.L. Prior, J.R. Clark, Flavonoid glycosides and antioxidant capacity of various blackberry, blueberry and red grape genotypes determined by high-performance liquid chromatography/mass spectrometry, *J. Sci. Food Agric.* 84 (2004) 1771–1782.
6. R.R. Huxley, H.A.W. Neil, The relation between dietary flavonol intake and coronary heart disease mortality: A meta-analysis of prospective cohort studies, *Eur. J. Clin. Nutr.* 57 (2003) 904–908.
7. K.J. Joshipura, F.B. Hu, J.E. Manson, M.J. Stampfer, E.B. Rimm, F.E. Speizer, G. Colditz, A. Ascherio, B. Rosner, D. Spiegelman, W.C. Willett, The effect of fruit and vegetable intake on risk for coronary heart disease, *Ann. Intern. Med.* 134 (2001) 1106–1114.
8. L.R. Howard, J.R. Clark, C. Brownmiller, Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season, *J. Sci. Food Agric.* 83 (2003) 1238–1247.
9. V. Lohachoompol, M. Mulholland, G. Srzednicki, J. Craske, Determination of anthocyanins in various cultivars of highbush and rabbiteye blueberries, *Food Chem.* 111 (2008) 249–254.
10. W. Kalt, C. Lawand, D.A.J. Ryan, J.E. McDonald, H. Donner, C.F. Forney, Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (*Vaccinium corymbosum* L.) during ripening and storage, *J. Amer. Soc. Hort. Sci.* 128 (2003) 917–923.
11. A.M. Connor, J.J. Luby, J.F. Hancock, S. Berkheimer, E.J. Hanson, Changes in fruit antioxidant activity among blueberry cultivars during cold-temperature storage, *J. Agric. Food Chem.* 50 (2002) 893–898.
12. R.A. Moyer, K.E. Hummer, C.E. Finn, B. Frei, R.E. Wrolstad, Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium*, *Rubus*, and *Ribes*, *J. Agric. Food Chem.* 50 (2002) 519–525.
13. H. Wang, G. Cao, R.L. Prior, Total antioxidant capacity of fruits, *J. Agric. Food Chem.* 44 (1996) 701–705.
14. M.K. Ehlenfeldt, R.L. Prior, Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry, *J. Agric. Food Chem.* 49 (2001) 2222–2227.
15. C.S. Ough, M.A. Amerine: *Methods for Analysis of Musts and Wines*, John Wiley & Sons Inc, Hoboken, NY, USA (1988).
16. V.L. Singleton, J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents, *Am. J. Enol. Vitic.* 16 (1965) 144–158.
17. X.P. Zhuang, Y.Y. Lu, G.S. Yang, Extraction and determination of flavonoid in ginkgo, *Chin. Herb. Med.* 23 (1992) 122–124.
18. H. Rebelein, Contribution to the determination of catechin contents into wine, *Deutsche Lebensm. Rundsch.* 61 (1965) 182–183 (in German).
19. C. Pompei, C. Peri, Determination of catechins in wines, *Vitis*, 9 (1971) 312–316.
20. P. Ribéreau-Gayon, E. Stonestreet, Determination of anthocyanins in red wine, *Bull. Soc. Chim. Fr.* 9 (1965) 2649–2652 (in French).
21. N. Pellegrini, D. Del Rio, B. Colombi, M. Bianchi, F. Brighenti, Application of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages, *J. Agric. Food Chem.* 51 (2003) 260–264.
22. W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, *LWT-Food Sci. Technol.* 28 (1995) 25–30.
23. I.F.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay, *Anal. Biochem.* 239 (1996) 70–76.
24. Y. Zheng, C.Y. Wang, S.Y. Wang, W. Zheng, Effect of high-oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity, *J. Agric. Food Chem.* 51 (2003) 7162–7169.
25. B. Lata, A. Trampczyńska, A. Mike, Effect of cultivar and harvest date on thiols, ascorbate acid and phenolic com-

- pounds content in blueberries, *Acta Sci. Pol.* 4 (2005) 163–171.
26. Climate Monitoring and Assessment for 2006, Meteorological and Hydrological Service, Zagreb, Croatia (2007) pp. 16–19.
 27. Climate Monitoring and Assessment for 2007, Meteorological and Hydrological Service, Zagreb, Croatia (2008).
 28. G. Giovanelli, S. Buratti, Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties, *Food Chem.* 112 (2009) 903–908.
 29. I. Koca, B. Karadeniz, Antioxidant properties of blackberry and blueberry fruits grown in the Black Sea Region of Turkey, *Sci. Hortic.* 121 (2009) 447–450.
 30. I. Ścibisz, M. Mitek, Antioxidant properties of highbush blueberry fruit cultivars, *EJPAU*, 10 (2007) 34.
 31. F. Shahidi, M. Naczyk: *Phenolics in Food and Nutraceuticals*, CRC Press, Boca Raton, FL, USA (2004).