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# Changes in berry quality of northern highbush blueberry (*Vaccinium corymbosum* L.) during the harvest season

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**Abstract:** As blueberries ripen successively and are commercially harvested over a period of several weeks, their composition may be altered during the harvest. The aim of this study was to compare blueberry fruit composition during the entire harvest season. Fully ripe fruits of two northern highbush blueberry cultivars (Bluecrop and Jersey) were harvested weekly at two different locations. Additionally, the Earliblue cultivar was monitored at a single location. Most of the examined characteristics changed during the harvest season and were cultivar-dependent, with smaller differences observed between sampling locations. Fruit weight of all cultivars decreased with the time of harvest. Levels of total sugars generally increased and total organic acids decreased, making the berries sweeter at the end of the growing period. Moreover, berries harvested later in the season generally contained more total anthocyanins. Total phenolic content generally decreased with successive harvests, mostly due to decreasing contents of total hydroxycinnamic acid derivatives and flavonol glycosides. The results indicate that blueberries harvested at later periods are smaller and contain comparable or increased levels of selected metabolites than larger fruits collected at the beginning of the harvest season.

**Key words:** Anthocyanins, fruit weight, primary metabolites, phenolics, seasonal variation, *Vaccinium*

## 1. Introduction

Highbush blueberry (*Vaccinium corymbosum* L.) fruits are a rich source of important secondary metabolites such as anthocyanins, flavanols, flavonols, and phenolic acids, like hydroxycinnamic acids (Gavrilova et al., 2011; Rodriguez-Mateos et al., 2012; Veberic et al., 2015). Due to the high nutritional value and balanced taste, blueberry consumption is continuously rising.

Blueberry plants are known for their successive ripening and berries are commercially harvested weekly over a period of 3 to 5 or more weeks. Blueberry fruiting season depends on the production region and cultivar and normally extends from late June until early September. During this period different growing conditions vary and may therefore significantly affect the fruit composition. It is well known that the phenolic profile of blueberries and other fruits is influenced by growing location and climatic conditions, such as soil nutrient content, water availability, day and night temperatures, and duration and intensity of sunlight (Howard et al., 2003; Uleberg et al., 2012; Zoratti et al., 2015a, 2015b). Additionally, genetic factors (cultivar characteristics) and maturity stage substantially determine the level of metabolites in fruit (Prior et al., 1998; Çelik et al., 2008; Ribera et al., 2010; Gündüz et al., 2015). The effect of fruit maturation and ripening on development and changes of phenolic compounds in blueberry and other *Vaccinium* sp. has previously been documented (Jaakola et al., 2002; Kalt et al., 2003; Castrejón et al., 2008; Çelik et al., 2008; Ozgen et al., 2008), but little information on seasonal variation in ripe blueberry fruit composition exists.

Bett-Garber et al. (2015) reported that blueberry fruits harvested 2 weeks apart differed in sweet/sour/bitter taste. Similar observations were reported by Du et al. (2011), who demonstrated that the volatile content and composition of blueberry fruits are moderately influenced by harvest date, environmental conditions, and growing location, but highlighted the impact of the cultivar. In addition to the effect of the latter, Łata et al. (2005) confirmed a significant influence of consecutive harvest dates within the same season on the level of selected blueberry phenolics and thiols as well as modified enzyme activity. In other small fruits like strawberry and aronia, weekly variation in flavor components (Jouquand et al., 2008), berry juice polyphenols, sugars, and antioxidant activity have been measured (Bolling et al., 2015). Similarly, distinct contents of specific phenolic compounds have been reported at

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different samplings of blackcurrant leaves during the vegetation period (Vagiri et al., 2015).

Because blueberry ripening is successive, the aims of the present study were: 1) to characterize the change in fruit quality characteristics, particularly berry fresh weight, sugars, organic acids, pH value, and polyphenolic profile, during the harvest season; 2) determine the differences among the selected cultivars; and 3) determine the differences between sampling locations. To our knowledge there has been no research on continuous modification of the blueberry phenolic profile throughout the harvest period, although the growing season reportedly affects the organoleptic properties as well as the phytonutrient composition of fruit. To optimize fruit quality and nutritional value of natural products, it is important to understand the variations of these components during the growing season.

## 2. Materials and methods

### 2.1. Plant material

Northern highbush blueberry fruits of three traditionally cultivated cultivars in Slovenia, Bluecrop, Jersey, and Earliblue, were hand-harvested from the experimental station of the Agricultural Institute of Slovenia, located at Brdo pri Lukovici (BPL) (46°10′9.34″N, 14°41′3.84″E, 370 m altitude). Bluecrop and Jersey cultivars were also harvested from a local blueberry orchard in Drenov grič (DG) (45°59′50.73″N, 14°19′55.28″E, 290 m altitude). Berries from both locations were harvested at the fully ripe stage in 2014. To ensure that all samples were equally ripe, blueberries were harvested when fruit skin color was completely blue, with lightness (L) values between 25.0 and 33.0, chroma (C) of 5.0–9.0, and hue angle (h°) of 265.0–280.5. In BPL the first harvest date was on 19 June for the Earliblue cultivar. Jersey was picked on 26 June at both locations and Bluecrop on 2 July at BPL and on 26 June at DG. Berries were harvested weekly until the end of the season, with the last harvest on 21 July in BPL and 1 August in DG.

Blueberries at both locations were cultivated in a bush training system with row spacing of 3 m × 1.2 m (BPL) or 3 m × 1.5 m (DG) and equipped with a drip irrigation system. Soil texture at BPL is silty loam with pH value of 4.1 and mineral composition of 15 mg $P_2O_5$ and 19 mg $K_2O$/100 g. Soil type at DG is peat, with high organic matter, pH value of 3.5, and mineral composition of 37 mg $P_2O_5$ and 22 mg $K_2O$/100 g (2014 soil analysis).

Only undamaged fruits were selected for the analysis. Immediately after harvest fruit weights and pH values of juice were measured and 500 g of fruit (from 10 plants) per cultivar and sampling date from each location was frozen in liquid nitrogen and stored for up to 1 month at –20 °C until chemical analyses.

### 2.2. Meteorological data

Daily temperatures (average and maximum) and precipitation were recorded at two locations (BPL and DG) during the span of the berry growing season (from June to August 2014). Data were obtained from the Slovenian Environment Agency (ARSO) and are presented in Figures 1 and 2.

### 2.3. Fruit characteristics

Fruit weight and pH value were recorded in five replications, each containing 20 fruits. pH value was evaluated in freshly extracted blueberry juice and was measured with a pH-meter (WTW, Weilheim, Germany).

### 2.4. Extraction and determination of sugars and organic acids

Primary metabolites (sugars and organic acids) were analyzed in whole berry fruits. For each blueberry cultivar from both locations and individual sampling dates, five

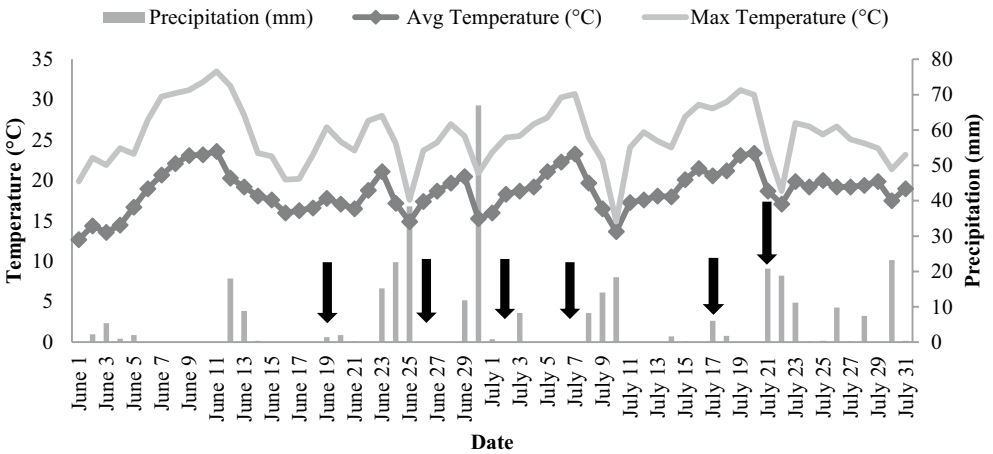


**Figure 1.** Average and maximum daily temperature (°C) and precipitation (mm) during berry ripening (1 June–31 July 2014) in the BPL location. The harvest dates are represented by black arrows.

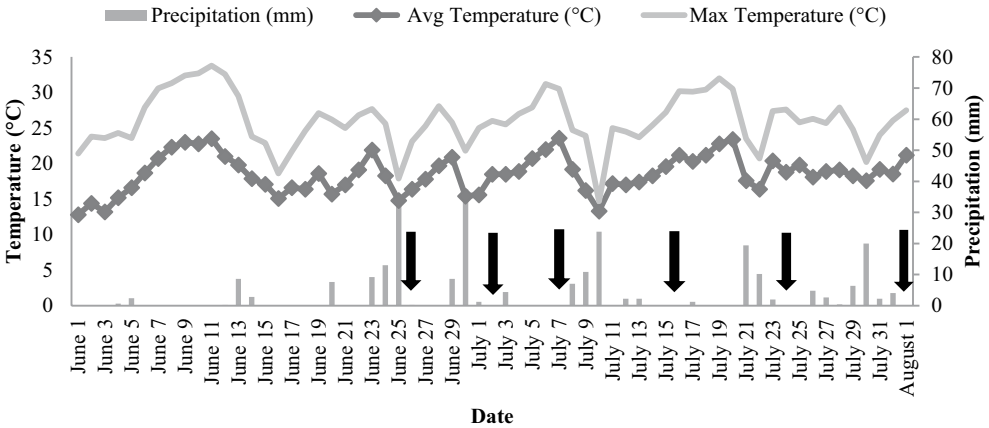


**Figure 2.** Average and maximum daily temperature (°C) and precipitation (mm) during berry ripening (1 June–1 August 2014) in the DG location. The harvest dates are represented by black arrows.

replications were carried out; each replication included several fruits. For the extraction of primary metabolites, 4 g of fruit was ground to a fine paste in a mortar, homogenized with 16 mL of double-distilled water, and left for 30 min at room temperature with continuous stirring. After the extraction, the methods followed procedures reported by Mikulic-Petkovsek et al. (2012). Sugars and organic acids content levels were expressed in mg $g^{-1}$ fresh weight (FW) of blueberries. Total analyzed sugars and organic acids were used for the determination of sugar/organic acid ratio.

### 2.5. Extraction and determination of phenolic compounds using HPLC–DAD–$MS^n$ analysis

As for sugars and organic acids, five replications (each included several fruits) were carried out for each cultivar from both locations and individual sampling dates for phenolic compounds determination. Berries were ground to a fine paste in a mortar chilled with liquid nitrogen and 3 g was extracted with 12 mL of methanol containing 3% (v/v) formic acid in a cooled ultrasonic bath for 1 h. After extraction, the fruit extracts were centrifuged at 9700 × *g* for 7 min at 4 °C, and the supernatant was filtered through a 0.2-µm Chromafil AO-45/25 polyamide filter (Macherey-Nagel) into a vial pending analysis (Mikulic-Petkovsek et al., 2015). Phenolic compounds were analyzed on a Thermo Finnigan Surveyor HPLC system (Thermo Fisher Scientific) with a diode array detector at 280 nm (flavanols, hydroxycinnamic and hydroxybenzoic acid derivatives), 350 nm (flavonols), and 530 nm (anthocyanins). The procedures were described previously by Mikulic-Petkovsek et al. (2015). Concentrations of phenolic compounds were calculated from peak areas of the sample and the corresponding standards and expressed in mg 100 $g^{-1}$ FW of blueberries. For compounds lacking standards, quantification was carried out using similar compounds as standards.

### 2.6. Determination of total phenolic content

The extraction of berry samples for the determination of total phenolic content (TPC) was done according to the same protocol as for individual phenolics. TPC of extracts was assessed by the Folin–Ciocalteu phenol reagent method of Singleton et al. (1999). To 100 µL of the sample extracts (diluted 1:3 (v/v) with MeOH), 6 mL of double-distilled water and 500 µL of Folin–Ciocalteu reagent were added, following the methodology of Mikulic-Petkovsek et al. (2015). TPC was expressed as gallic acid equivalents (GAE) in mg 100 $g^{-1}$ FW of blueberries. Absorption was measured in five replications.

### 2.7. Chemicals

The following standards were used for the determination of sugars and organic acids: fructose, glucose, and sucrose; citric and malic acid from Fluka Chemie (Buchs, Switzerland), and quinic and shikimic acid from Sigma-Aldrich Chemie (Steinheim, Germany). The following standards were used for the quantification of phenolic compounds: chlorogenic acid (5-caffeoylquinic acid), neochlorogenic acid (3-caffeoylquinic acid), cyanidin-3-glucoside, ellagic acid, and malvidin-3-glucoside from Sigma-Aldrich Chemie; caffeic and ferulic acids and (+)-catechin from Roth (Karlsruhe, Germany), (–)-epicatechin, quercetin-3-rhamnoside, quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rutinoside, *p*-coumaric acid, procyanidin B2, cyanidin-3-galactoside, and kaempferol from Fluka Chemie; quercetin-3-xyloside, quercetin-3-arabinopyranoside, and myricetin-3-rhamnoside from Apin Chemicals (Abingdon, UK); and isorhamnetin-3-glucoside, delphinidin-3-glucoside, petunidin, and peonidin-3-glucoside from Extrasynthese (Genay, France). Methanol for extraction of phenolics was acquired from Sigma. The chemicals for the mobile phases were HPLC–MS grade acetonitrile and formic acid from Fluka Chemie. Water for

the mobile phase was double distilled and purified with the Milli-Q system (Millipore, Bedford, MA, USA). For the total phenolic content, Folin–Ciocalteu phenol reagent (Fluka Chemie), sodium carbonate (Merck, Darmstadt, Germany), gallic acid (Sigma-Aldrich Chemie), and ethanol (Sigma-Aldrich Chemie) were used.

### 2.8. Statistical analysis

Results were evaluated with the StatGraphics Plus 4.0 program (Manugistics, Rockville, MD, USA). Data from all of the analyses were tested for any differences among harvest dates within each cultivar (data from the BPL location) and between sampling locations (average values combining data of all harvest dates) using one-way analysis of variance (ANOVA). The differences were tested using the Duncan test for seasonal changes and LSD test for locations with a significance level of 0.05. Statistical differences among cultivars (average values combining data of all harvest dates from the BPL location) were also tested with ANOVA and Duncan tests and are commented on in the text; however, the data are not presented in table form.

## 3. Results and discussion

### 3.1. Blueberry fruit characteristics and the content of sugars and organic acids

Changes of berry characteristics and primary metabolite content during the harvest season from the BPL sampling location are presented in Table 1. In Table 2, differences in average values combining data of all harvest dates are displayed between the two sampling locations (BPL and DG). Fruit weight of all blueberry cultivars gradually decreased with almost every successive harvest (Table 1). On the other hand, no significant differences in average berry weight were recorded between the sampling locations (Table 2). On the first sampling date, berries were from 16% (Bluecrop) to 53% (Earliblue) larger compared to the last picking date. It is widely known that fruit weight increases during maturation and ripening (Kalt et al., 2003; Ribera et al., 2010), but as all berries were sampled at a comparable maturity stage the differences cannot be ascribed to these factors. Similar to our findings, Castrejón et al. (2008) reported an average 12% decrease of fruit weight of selected blueberry cultivars after reaching

**Table 1.** Fruit fresh weight (g), content levels of individual and total sugars and organic acids (mg $g^{-1}$ FW), sugars/organic acids ratio, and pH value of selected blueberry cultivars determined at successive harvest dates from the BPL location.

| | Fruit weight | Glucose | Fructose | Total sugars | Citric acid | Malic acid | Quinic acid | Total organic acids | Ratio sugars/ acids | pH |
|---|---|---|---|---|---|---|---|---|---|---|
| Bluecrop | | | | | | | | | | |
| H1 | 1.90 ± 0.02a | 25.55 ± 0.28b | 26.04 ± 0.29c | 53.49 ± 0.56c | 11.76 ± 0.66a | 0.55 ± 0.04a | 3.37 ± 0.24 | 15.68 ± 0.96a | 3.29 ± 0.16c | 2.82 ± 0.03 |
| H2 | 1.91 ± 0.05a | 30.68 ± 1.91a | 30.81 ± 1.91b | 61.49 ± 3.83b | 11.16 ± 0.77a | 0.38 ± 0.02b | 3.72 ± 0.26 | 15.26 ± 0.49ab | 4.03 ± 0.18bc | 2.87 ± 0.05 |
| H3 | 1.86 ± 0.03a | 31.69 ± 0.66a | 31.95 ± 1.36ab | 63.64 ± 1.34ab | 10.94 ± 0.67ab | 0.35 ± 0.02b | 3.82 ± 0.46 | 15.11 ± 0.84ab | 4.21 ± 0.24b | 2.85 ± 0.02 |
| H4 | 1.64 ± 0.03b | 34.11 ± 0.86a | 34.78 ± 0.89a | 68.89 ± 1.75a | 8.94 ± 0.57c | 0.36 ± 0.02b | 3.90 ± 0.12 | 13.19 ± 0.64b | 5.22 ± 0.21a | 2.93 ± 0.04 |
| Earliblue | | | | | | | | | | |
| H1 | 2.00 ± 0.03a | 38.88 ± 1.13a | 40.27 ± 1.13ab | 79.15 ± 2.48ab | 4.22 ± 0.21b | 0.15 ± 0.01a | 3.85 ± 0.15a | 8.22 ± 0.33ab | 9.34 ± 0.34c | 3.50 ± 0.05bc |
| H2 | 1.50 ± 0.04b | 33.68 ± 0.25b | 35.17 ± 0.09c | 68.85 ± 0.33d | 5.35 ± 0.16a | 0.16 ± 0.01a | 3.48 ± 0.16ab | 8.99 ± 0.29a | 7.14 ± 0.10d | 3.12 ± 0.09d |
| H3 | 1.55 ± 0.03b | 39.06 ± 1.70a | 40.29 ± 1.70ab | 79.34 ± 3.40ab | 3.84 ± 0.14b | 0.13 ± 0.01a | 3.77 ± 0.80a | 7.73 ± 0.27b | 9.63 ± 0.17c | 3.40 ± 0.02c |
| H4 | 1.48 ± 0.03b | 38.12 ± 0.54a | 38.77 ± 0.67bc | 76.90 ± 0.91bc | 2.85 ± 0.37c | 0.12 ± 0.01a | 3.18 ± 0.14b | 6.15 ± 0.49c | 11.75 ± 0.74b | 3.71 ± 0.08ab |
| H5 | 1.43 ± 0.04bc | 34.22 ± 1.12b | 36.01 ± 1.06c | 70.23 ± 2.19cd | 2.04 ± 0.07d | 0.08 ± 0.01b | 2.62 ± 0.12c | 4.67 ± 0.19d | 13.61 ± 0.10a | 3.75 ± 0.13ab |
| H6 | 1.31 ± 0.05c | 41.68 ± 1.21a | 43.53 ± 1.30a | 85.21 ± 2.50a | 1.99 ± 0.27d | 0.06 ± 0.02b | 3.14 ± 0.12b | 4.86 ± 0.25d | 14.01 ± 0.85a | 3.89 ± 0.10a |
| Jersey | | | | | | | | | | |
| H1 | 1.62 ± 0.01a | 39.81 ± 1.39 | 39.28 ± 0.58 | 79.09 ± 2.79 | 8.41 ± 0.68a | 0.29 ± 0.02a | 3.91 ± 0.29 | 12.60 ± 0.94a | 6.11 ± 0.06b | 2.95 ± 0.03ab |
| H2 | 1.63 ± 0.04a | 40.35 ± 0.60 | 41.54 ± 0.62 | 81.89 ± 1.21 | 6.15 ± 0.11b | 0.24 ± 0.02ab | 3.67 ± 0.09 | 10.05 ± 0.17c | 8.15 ± 0.18a | 3.02 ± 0.07a |
| H3 | 1.49 ± 0.03b | 39.15 ± 1.56 | 41.83 ± 0.72 | 80.98 ± 3.18 | 7.76 ± 0.32ab | 0.22 ± 0.01b | 3.47 ± 0.13 | 11.45 ± 0.27ab | 6.96 ± 0.37ab | 2.76 ± 0.02c |
| H4 | 1.41 ± 0.01b | 40.76 ± 1.97 | 39.96 ± 1.12 | 80.72 ± 3.94 | 6.66 ± 0.95ab | 0.23 ± 0.01b | 3.61 ± 0.15 | 10.50 ± 0.87c | 8.09 ± 0.95a | 2.88 ± 0.02bc |
| H5 | 1.25 ± 0.30c | 42.10 ± 1.32 | 42.59 ± 1.24 | 84.69 ± 2.56 | 7.52 ± 0.53ab | 0.26 ± 0.01ab | 3.52 ± 0.07 | 11.30 ± 0.49ab | 7.96 ± 0.29a | 2.88 ± 0.05bc |

Numbers in the column caption of each cultivar represent the harvest date (H1–H6). Different letters in columns denote significant differences among sampling dates for each individual cultivar (Duncan's test, $P < 0.05$).

**Table 2.** Average fruit fresh weight (g), content levels of individual and total sugars and organic acids (mg $g^{-1}$ FW), sugars/organic acids ratio, and pH value calculated from data of all harvest dates separately for each blueberry cultivar and location.

| Location | Bluecrop | | Jersey | |
|---|---|---|---|---|
| | BPL | DG | BPL | DG |
| Fruit weight | 1.83 ± 0.03 | 1.82 ± 0.05 | 1.48 ± 0.04 | 1.47 ± 0.04 |
| Glucose | 30.51 ± 0.95 b | 46.26 ± 1.00 a | 40.43 ± 0.62 b | 48.53 ± 1.40 a |
| Fructose | 30.89 ± 0.85 b | 41.63 ± 0.88 a | 41.04 ± 0.60 | 44.24 ± 1.25 |
| Total sugars | 61.88 ± 1.90 b | 87.90 ± 1.88 a | 81.47 ± 1.22 b | 92.77 ± 2.64 a |
| Citric acid | 10.70 ± 0.41 a | 6.68 ± 0.51 b | 7.30 ± 0.30 a | 5.39 ± 0.38 b |
| Malic acid | 0.41 ± 0.02 a | 0.22 ± 0.01 b | 0.25 ± 0.01 a | 0.21 ± 0.01 b |
| Quinic acid | 3.70 ± 0.11 a | 3.27 ± 0.10 b | 3.64 ± 0.07 a | 3.16 ± 0.08 b |
| Total organic acids | 14.81 ± 0.45 a | 10.17 ± 0.55 b | 11.18 ± 0.32 a | 8.76 ± 0.33 b |
| Ratio sugars/acids | 4.19 ± 0.21 b | 9.39 ± 0.79 a | 7.45 ± 0.26 b | 11.14 ± 0.68 a |
| pH | 2.87 ± 0.02 b | 3.13 ± 0.06 a | 2.90 ± 0.03 b | 3.23 ± 0.05 a |

Letters in the row caption represent the sampling location (BPL and DG). Different letters in rows denote significant differences among sampling locations for each individual cultivar (LSD test, $P < 0.05$).

technological maturation and throughout the successive harvest. Reduced berry weight during the successive harvest in the present study can potentially be linked to high temperatures and increased solar radiation at the end of the harvest season, impacting plant water status and reducing photosynthesis (Kumudini, 2004). Bluecrop blueberries from the BPL location were characterized by the highest fruit weight; this cultivar yielded 19%–23% larger fruit compared to Earliblue and Jersey cultivars. Bluecrop reportedly develops large berries (Castrejón et al., 2008) favored by producers (easier hand-harvesting) and consumers (attractive fruit size).

Although all blueberries were harvested at a technologically ripe stage, differences in the content of sugars and organic acids were detected among harvest dates and cultivars and also between the two growing locations (Tables 1 and 2). Sugars and organic acids have an important impact on the sensory quality of fruit, which might also change blueberry taste during the harvest season. Corresponding to the research of Milivojevic et al. (2012), glucose and fructose were the prevalent sugars in all blueberry samples. In addition to these two sugars, traces of sucrose have also been detected in fruit of all blueberry cultivars (data not shown). The content of individual and total sugars generally increased during the successive harvest, with the exception of the Jersey cultivar. Nevertheless, the highest content of total sugars was measured in fruit on the last harvest date, regardless of the cultivar. The highest total sugars increase (29%) between the first and the last harvest week was recorded in Bluecrop fruit. In accordance with our study, Uleberg et al. (2012) observed increased contents of fructose and glucose in different bilberry (*Vaccinium myrtillus* L.) clones during the course of the harvest period. This pattern has been attributed to less mature/not fully ripe berries at the beginning of the season and/or increased sugar content with the progression of the ripening process. Highest individual and total sugar contents were measured in Jersey blueberries and the lowest in Bluecrop fruit.

As previously reported (Mikulic-Petkovsek et al., 2012; Milivojevic et al., 2012), citric acid was the most abundant organic acid in all blueberry samples, followed by quinic acid. These two acids combined accounted for approximately 97% of total organic acids in blueberry fruit (Table 1). Malic acid represented less than 3% of total organic acids, while shikimic acid was only detected in traces (data not shown). The content levels of total organic acids decreased by between 19% (Bluecrop) and 69% (Earliblue) from the first to the last harvest date, but the decrease was inconsistent in fruit of the Jersey cultivar. Consequently, the sugars/organic acids ratio, which is used as an index of sweetness, was lower at the beginning of harvest, indicating a sweeter taste of berries collected on the last sampling dates. Changes in the content of total sugars and organic acids during the blueberry harvest period were comparable to results of studies monitoring the effect of fruit ripening on the accumulation of primary metabolites (Siriwoharn et al., 2004; Mikulic-Petkovsek

et al., 2012). However, berries in the present study were collected at the fully ripe stage on all sampling dates. Correspondingly, Bolling et al. (2015) reported a decrease in organic acid contents of aronia juice from the first to the last harvest date, which contributed to a higher Brix/organic acid ratio at the end of the growing season. The highest total organic acids were measured in Bluecrop berries and the lowest in Earliblue fruit, which was also characterized by a particularly high sugars/organic acids ratio. Conversely, Bluecrop blueberries were distinguished by an extremely low ratio. Opposite to the average level of total sugars, which was 13%–43% higher at the DG location, average total organic acids were 28%–46% higher at BPL, suggesting that blueberry fruits from DG taste sweeter compared to fruits from BPL. Differences in the content of primary metabolites between the two locations could be ascribed to factors such as soil fertility, agricultural practices, and slightly different environmental conditions.

The pH value of Bluecrop juice was unaffected (Table 1) by the harvest date. However, the pH value of Jersey juice decreased from the second to the third harvest and an increase of the pH value of Earliblue juice was recorded from the second-to-last harvest. On the other hand, Bolling et al. (2015) did not detect any significant changes in aronia juice pH values during 7 weeks of harvest. Similarly, pH values of different small fruits reportedly remain constant or increase slightly with the progression of fruit maturity (Mikulic-Petkovsek et al., 2015). The highest pH value was measured in Earliblue blueberry juice. Blueberries from the DG location were generally characterized by higher average pH values compared to the BPL location.

### 3.2. Phenolic compound composition

Differences in the content of total anthocyanins (TA), hydroxycinnamic (HCA) and hydroxybenzoic (HBA) acid derivatives, flavonols, flavanols, and total phenolic content (TPC) were determined among harvest dates from the BPL sampling location (Table 3). Differences between the BPL and DG sampling locations are presented in average values combining data of all harvest dates (Table 4). Bluecrop fruit from the BPL location was characterized by higher average content of total flavanols compared to the berries harvested at the DG site. Conversely, Jersey blueberries from the DG location accumulated more HCA

**Table 3.** Content levels of total anthocyanins, hydroxycinnamic acid derivatives (HCA), flavonols, flavanols, hydroxybenzoic acid derivatives (HBA), and total phenolic acids (TPC) (mg 100 $g^{-1}$ FW) of selected blueberry cultivars determined at successive harvest dates from the BPL location.

| | Total anthocyanins | Total HCA | Total flavonols | Total flavanols | Total HBA | TPC |
|---|---|---|---|---|---|---|
| Bluecrop | | | | | | |
| H1 | 103.0 ± 6.98 b | 187.1 ± 6.83 a | 30.43 ± 0.72 a | 32.50 ± 0.58 | 10.13 ± 0.90 | 347.0 ± 10.52 a |
| H2 | 117.7 ± 5.69 ab | 149.7 ± 6.26 b | 26.75 ± 0.79 ab | 34.03 ± 1.52 | 9.49 ± 0.27 | 304.0 ± 5.59 ab |
| H3 | 132.5 ± 8.94 a | 130.8 ± 14.5 b | 23.83 ± 2.46 b | 41.75 ± 4.93 | 8.65 ± 0.83 | 294.2 ± 21.32 b |
| H4 | 126.7 ± 12.4 ab | 129.7 ± 4.08 b | 22.71 ± 1.36 b | 39.71 ± 4.93 | 9.19 ± 0.59 | 305.1 ± 1.71 ab |
| Earliblue | | | | | | |
| H1 | 171.0 ± 9.78 | 113.4 ± 4.65 b | 28.09 ± 1.52 ab | 45.28 ± 2.44 ab | 9.34 ± 0.58 a | 301.7 ± 16.0 ab |
| H2 | 172.8 ± 5.49 | 150.6 ± 7.31 a | 31.13 ± 1.00 a | 55.39 ± 1.00 a | 7.95 ± 0.47 ab | 322.2 ± 6.28 a |
| H3 | 186.1 ± 14.5 | 114.2 ± 9.24 b | 25.85 ± 2.83 ab | 51.16 ± 3.58 ab | 7.52 ± 0.22 b | 300.8 ± 15.9 ab |
| H4 | 173.3 ± 5.76 | 104.5 ± 5.22 bc | 24.86 ± 1.33 ab | 48.51 ± 3.03 ab | 8.16 ± 0.83 ab | 273.0 ± 10.1 c |
| H5 | 167.0 ± 18.7 | 98.47 ± 4.62 bc | 21.89 ± 3.24 b | 43.15 ± 5.07 b | 8.07 ± 0.63 ab | 263.7 ± 19.0 c |
| H6 | 192.7 ± 10.4 | 87.22 ± 3.48 c | 21.32 ± 2.26 b | 41.72 ± 1.52 b | 8.75 ± 0.79 ab | 262.9 ± 12.1 c |
| Jersey | | | | | | |
| H1 | 187.4 ± 6.45 b | 94.36 ± 1.58 a | 24.02 ± 1.03 a | 30.73 ± 1.92 | 7.54 ± 0.48 | 250.9 ± 8.08 ab |
| H2 | 174.7 ± 4.07 b | 81.18 ± 2.21 bc | 21.09 ± 0.28 ab | 31.04 ± 1.30 | 8.26 ± 0.30 | 239.3 ± 7.86 b |
| H3 | 241.9 ± 11.6 a | 91.16 ± 1.22 ab | 23.49 ± 1.34 a | 39.19 ± 1.64 | 8.59 ± 0.36 | 272.4 ± 3.63 a |
| H4 | 228.8 ± 14.6 a | 78.47 ± 3.68 c | 17.62 ± 1.63 b | 35.86 ± 6.67 | 8.65 ± 0.14 | 258.6 ± 9.59 ab |
| H5 | 234.0 ± 9.41 a | 79.66 ± 8.93 c | 21.10 ± 0.68 ab | 36.80 ± 2.20 | 8.36 ± 0.83 | 265.8 ± 2.03 a |

Numbers in the column caption of each cultivar represent the harvest date (H1–H6). Different letters in columns denote significant differences among sampling dates for each individual cultivar (Duncan's test, $P < 0.05$).

**Table 4.** Average content levels of total anthocyanins, hydroxycinnamic acid derivatives (HCA), flavonols, flavanols, hydroxybenzoic acid derivatives (HBA), and total phenolic content (TPC) (mg 100 $g^{-1}$ FW) calculated from data of all harvest dates separately for each blueberry cultivar and location.

| Location | Bluecrop | | Jersey | |
|---|---|---|---|---|
| | BPL | DG | BPL | DG |
| Total anthocyanins | 120.0 ± 4.91 | 133.1 ± 5.97 | 213.4 ± 7.68 | 210.3 ± 10.6 |
| Total HCA | 149.3 ± 7.52 | 140.8 ± 6.16 | 84.97 ± 2.14 b | 110.7 ± 5.42 a |
| Total flavonols | 25.93 ± 1.06 | 27.65 ± 1.51 | 21.46 ± 0.68 | 23.31 ± 0.62 |
| Total flavanols | 37.00 ± 1.90 a | 31.27 ± 2.61 b | 34.72 ± 1.37 | 36.20 ± 2.46 |
| Total HBA | 9.37 ± 0.27 | 9.31 ± 0.61 | 8.28 ± 0.22 | 8.09 ± 0.49 |
| TPC | 312.6 ± 8.90 | 290.8 ± 10.0 | 257.3 ± 4.00 b | 291.7 ± 4.69 a |

Letters in the row caption represent the sampling location (BPL and DG). Different letters in rows denote significant differences among sampling locations for each individual cultivar (LSD test, $P < 0.05$).

and TPC. The differences can be attributed to diverse growing conditions.

Anthocyanins were the predominant phenolic group in highbush blueberry cultivars (except Bluecrop). TA content was in range of 103.0–241.9 mg 100 $g^{-1}$ FW and constituted 35%–55% of total analyzed phenolics (TAP) in blueberry fruit. The highest proportion of TA included delphinidin and malvidin glycosides, followed by petunidin, cyanidin, and peonidin glycosides, as reported by Veberic et al. (2015) (data not shown). Significant differences in TA levels were detected among cultivars and during the successive harvest. However, TA content of Earliblue blueberries did not change during the course of sampling. Anthocyanins increase during fruit maturation and well-matured blueberries accumulate higher levels of TA (Prior et al., 1998; Kalt et al., 2003; Castrejón et al., 2008). However, analyzed berries in our study were fully ripe, suggesting that differences were caused by other factors. TA content of the Bluecrop cultivar increased by 29% from the first to the third harvest date. Similarly, lower levels of TA were measured on the first two sampling dates in the Jersey cultivar. TA increase has been reported in the first 5 weeks of aronia ripening (Bolling et al., 2015), with a slight decline of TA at subsequent samplings. A 34.5% increase of TA was also measured by Castrejón et al. (2008) in Bluecrop and other blueberry cultivars from the first to the third harvest date. The authors linked the decrease with diminished berry weight as TA content is reportedly affected by berry size (Howard et al., 2003; Castrejón et al., 2008). This relationship was also confirmed in our research, as the lowest TA content was measured in the cultivar with the highest berry weight (Bluecrop). Conversely, Jersey was characterized by smaller berries and the highest TA content. Correspondingly, large first-time harvested berries contained lower TA levels. Genetic and maturity factors influence the ability of blueberries to synthesize anthocyanins (Howard et al., 2003; Uleberg et al., 2012), but variations in TA content between harvest dates in our study were probably caused by environmental seasonal differences, especially temperature. In addition to light intensity and soil conditions, temperature was the major factor affecting anthocyanin content and composition in *Vaccinium* berries in studies by Zoratti et al. (2015a, 2015b). TA content radically declined in fruit when the maximum daily temperature exceeded 35 °C (Mori et al., 2007; Zoratti et al., 2015a). This is potentially the limiting factor in uniform color development during blueberry fruit ripening, although fruit color characteristics were unaffected in the present study.

Higher levels of total HCA compared to TA were only measured in the Bluecrop cultivar, which is in accordance with the results of Castrejón et al. (2008), who reported HCA as the most prevalent phenolic subclass during all stages of blueberry ripening. Conversely, other authors defined them as the second predominant group, subsequent to anthocyanins (Kalt et al., 2003; Gavrilova et al., 2011). Differences can probably be ascribed to dissimilar maturity stages among berries and diverse cultivars analyzed. Bluecrop blueberries accumulated the highest total HCA levels (44% of TAP) and, on the contrary, the lowest total HCA levels were measured in Jersey fruit (25% of TAP). Earliblue blueberries contained moderate levels of total HCA. The results correspond to data reported by Gavrilova et al. (2011), although our values were somewhat higher. Chlorogenic acid was the predominant phenolic acid throughout the growing season and in all blueberry cultivars, followed by ferulic, caffeic, and *p*-coumaric acid derivatives (data not shown).

Similar HCA profiles were reported previously (Gavrilova et al., 2011; Može et al., 2011; Gibson et al., 2013). As the harvest season progressed, total HCA decreased in all cultivars. Earliblue blueberries accumulated 73% less total HCA by the last harvest compared to the second harvest. Comparable levels of total HCA were measured in Jersey fruit, in which only a slight decrease (18%) from the first to the last two samplings was recorded. A 33% decrease of total HCA was observed in aronia juice during 7 weeks of harvest (Bolling et al., 2015). Similarly, HCA decreased by 27% in apple peel during 5 weeks of advanced ripening (Bizjak et al., 2013). Blueberry maturation and ripening also decrease the levels of HCA; unripe green blueberries contained significantly more HCA compared to ripe blue fruit (first harvest), although the contents were slightly higher on the last (third) harvest date in selected cultivars (Castrejón et al., 2008).

Total flavonol glycosides were present in lower quantities, accounting for 6%–8% of TAP compounds in blueberries, which corresponds to the results of Cho et al. (2005). Among total flavonol glycosides, quercetin-3-galactoside was the most abundant individual compound in blueberries, followed by other quercetin and myricetin glycosides as previously reported (Cho et al., 2005; Gavrilova et al., 2011; Gibson et al., 2013) (data not shown). Glycosides of kaempferol, laricitrin, isorhamnetin, and syringetin were additionally detected in blueberries and their occurrence corresponds with the report by Vrhovsek et al. (2012). Significant changes in total flavonol glycosides during the growing period were detected in all blueberry cultivars. The content of total flavonol glycosides generally decreased with successive harvest and a 34% and 46% decrease was measured in Bluecrop and Earliblue berries from the second harvest onwards. A similar pattern of flavonol decrease during blueberry maturation was observed by Gibson et al. (2013). Castrejón et al. (2008) also reported decreased flavonols levels in unripe green and unripe purple blueberries. Moreover, little or no flavonols were detected in ripe berries. Conversely, Cho et al. (2005) and Vrhovsek et al. (2012) detected between 13.70 and 32.75 mg 100 $g^{-1}$ FW of total flavonols in ripe blueberries, which is in accordance with our data. Jersey blueberries accumulated significantly lower levels of total flavonol glycosides compared to the other cultivars analyzed in the study.

Catechin, epicatechin, and different procyanidin oligomers were identified from the group of flavanols, combined representing 10%–13% of TAP (data not shown). Total flavanols were significantly affected by harvest only in the Earliblue cultivar; blueberries contained 33% more total flavanols at the second harvest compared to the last two sampling weeks. Zifkin et al. (2012) observed a decline in total proanthocyanidin levels as the blueberry fruits matured. In contrast, aronia proanthocyanidins increased between the first and seventh week of harvest. In different *Ribes* spp., diverse patterns of flavanol turnover during advanced ripening were reported by Mikulic-Petkovsek et al. (2015). Rodriguez-Mateos et al. (2012) observed a considerable variation in total flavanol content among different blueberry varieties. In the present study, a significantly higher content of total flavanols was measured in Earliblue blueberries but the other two cultivars contained comparable levels.

Depside, a hydroxybenzoic acid polymer, was quantified in low amounts, representing 2%–3% TAP. Häkkinen et al. (1999) reported low levels of hydroxybenzoic acids in berries of the genera *Ribes*, *Fragaria*, *Rubus*, and *Vaccinium*, and no hydroxybenzoic acids in blueberry. Differences in depside contents among successive harvests were only significant in the Earliblue cultivar. Blueberries of the latter cultivar contained higher levels of depside with the progression of sampling (subsequent to the third harvest). Bluecrop fruits contained the highest levels of depside, while the other two cultivars accumulated comparable amounts.

It is well known that TPC of blueberries and other fruits is strongly influenced by cultivar and growing conditions and also by the degree of ripeness (Kalt et al., 2003; Siriwoharn et al., 2004; Mikulic-Petkovsek et al., 2015). Significant differences in TPC were detected among blueberry cultivars and harvest dates in the present study. The highest TPC was measured in Bluecrop berries (312.6 mg 100 $g^{-1}$ FW) and the lowest in Jersey fruit (257.3 mg 100 $g^{-1}$ FW), with similar results reported by Moyer et al. (2002). TPC of Bluecrop blueberries decreased by 18% from the first to the third harvest and TPC of Earliblue cultivar by 25% from the second harvest on. The TPC level of Jersey cultivar was somewhat higher at the third and last sampling compared to the second harvest date; however, no clear seasonal pattern was observed for this cultivar. Siriwoharn et al. (2004) and Gibson et al. (2013) essentially reported a constant TPC of blueberries and blackberries during fruit maturation, although a slight decrease was recorded in ripe berries compared to unripe fruit. Moreover, an increase in TPC was measured in overripe blackberry fruits in comparison to berries at technological maturity. A prominent TPC decrease was also measured in ripe blueberries opposed to unripe, green fruit (Kalt et al., 2003; Castrejón et al., 2008). A decline was contributed to the shift in the pool of TPC towards anthocyanins. As in our study, Castrejón et al. (2008) detected a different response of blueberry cultivars to successive picking of fully ripe berries.

In conclusion, our results showed that compositions of sugars, organic acids, and phenolic compounds mostly varied during the successive harvest and among cultivars.

Distinct differences among cultivars in all examined characteristics may reflect their different genetic origins. Although cultivar was the major factor influencing blueberry fruit composition, different environmental conditions affected synthesis and accumulation of biochemical compounds during harvest. Only minor differences in fruit quality (mostly in the levels of primary metabolites) were determined between sampling locations. These can be ascribed to differences in soil properties and agricultural practices as microclimatic conditions were not dissimilar. Our results could represent important information for producers, since the study showed that later harvested blueberries were smaller, but on the other hand of equal or superior phytochemical quality compared to larger fruits harvested at the beginning of the growing season.

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