Study of the physicochemical properties of highbush blueberry and wild bilberry fruit in central Bosnia

Jasmina ALIMAN1,* Izabela MICHALAK2, Edin BUŠATLIĆ1, Lamija ALIMAN3, Mirko KULINA4, Mirjana RADOVIĆ4, Jasna HASANBEGOVIĆ1

1Agro Mediterranean Faculty, “Džemal Bijedić” University of Mostar, Mostar, Bosnia and Herzegovina
2Department of Advanced Material Technologies, Faculty of Chemistry, Wrocław University of Science and Technology, Wrocław, Poland
3Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
4Faculty of Agriculture, University of East Sarajevo, Istočno Novo Sarajevo, Bosnia and Herzegovina

Abstract: Although around the globe numerous studies have been conducted on the nutritional composition of blueberry/bilberry and its effect on human health, very little is known about the fruit in Bosnia and Herzegovina. This is the first report regarding the physical and chemical characteristics of the cultivated highbush blueberry and bilberry harvested in the same climatic conditions in Bosnia and Herzegovina. In this paper we present the physical (weight) and chemical (soluble solids content, total sugars, titratable acidity, pH, and total phenols) properties of the wild bilberry (Vaccinium myrtillus L.) and cultivated highbush—‘Earliblue’, ‘Bluegold’, ‘Bluecrop’, ‘Goldtraube’—blueberry (Vaccinium corymbosum L.). Among the highbush blueberry cultivars, ‘Bluegold’ (2.07–2.11 g) and ‘Bluecrop’ (2.08–2.11 g) had the highest weight. ‘Bluecrop’ had the highest soluble solids content (13.3–13.7 °Brix) and total sugars (9.73%–9.94%), but the lowest content of titratable acidity (0.70–0.72 g/100 g), which was highest in ‘Goldtraube’ (0.92–0.93 g/100 g). This cultivar also had the highest content of total phenols (289–309 mg gallic acid equivalent/100 g of fresh weight). In the case of wild bilberry, the following values were obtained: average weight 0.28–0.32 g, soluble solids content 11.1–11.3 °Brix, total sugars 7.23%–7.37%, titratable acidity 0.99%–1.02%, and total phenols 431–455 mg/100 g. Blueberry and wild bilberry also had a fairly uniform pH (3.2–3.6). All samples had a valuable composition and so it is advisory to expand their production in Bosnia and Herzegovina in order to increase their consumption.

Key words: Blueberry and wild bilberry, fruit weight, soluble solids content, titratable acidity, total sugars, total phenols

Received: 12.02.2019 • Accepted/Published Online: 19.09.2019 • Final Version: 01.04.2020

1. Introduction
The highbush blueberry (Vaccinium corymbosum L.) is grown in the United States, Canada, Chile, England, the Netherlands, Belgium, Germany, Switzerland, Poland, New Zealand, and Australia. The world’s blueberry production grew constantly from 262 t in 2006 to 552 t in 2016. The United States (269 t), Canada (179 t), Mexico (29 t), Poland (14.7 t), and Germany (10.7 t) are the top five producers (FAOSTAT, 2018). The Food and Agriculture Organization of the United Nations (FAOSTAT, 2018) states that Europe’s total blueberry production has been growing, so that in 2016 it stood at 65 t from 13 ha. Europe’s largest producers of blueberry are not harvesting enough to fully meet the demands of EU consumers and so the resultant lack is met by imports from other continents.

The production of this fruit has been experiencing expansion in the last decade, boosted by the results of numerous studies that proved that blueberry has a very favorable impact on human health (Prior et al., 1998; Wu et al., 2002; Scalbert et al., 2005; Crawford and Mellentin, 2008; Scalzo et al., 2008; Basu et al., 2010; Krikorian et al., 2010; Carey et al., 2014; Nil and Park, 2014; Singh, 2018). Its antioxidant (Prior et al., 1998; Sellappan et al., 2002) and antiinflammatory activities (Lau et al., 2007) have been shown to be responsible for the reduction of metabolic diseases (Esposito et al., 2014). The consumption of whole blueberries improved insulin sensitivity (Stull et al., 2010). Basu et al. (2010) showed cardioprotective effects of blueberry in improving features of the metabolic syndrome. Del Bo’ et al. (2013) referred to the effect of 300 g of blueberry intake on selected markers of oxidative stress and antioxidant protection.

Blueberry contains a large number of organic and inorganic compounds, the content of which varies depending on the cultivar, environmental characteristics, agricultural technology, fruit maturity, and the abundance...
of the genus (Häkkinen and Törrönen, 2010; Gündüz et al., 2015). Fresh blueberries contain water (84%), carbohydrates (9.7%), proteins (0.6%), and fat (0.4%) (Michalska and Łysiak, 2015), and offer significant health benefits because of their high levels of polyphenols, antioxidants, vitamins, minerals, and fiber (Zhao, 2007).

It is especially phenolic compounds—that favorably affect the health of people in that they decrease the frequency of the incidence of cardiovascular disease, diabetes, and cancer (Stevenson and Lowe, 2009; Adams et al., 2010; Samad et al., 2014). Blueberry’s phytochemicals may inhibit the growth and potential metastasis of breast and colon cancer (Adams et al., 2010; Samad et al., 2014). The content of total phenols, which is more influenced by the genotype than by environmental conditions, is an important antioxidant (Connor et al., 2002; Howard et al., 2003; Gündüz et al., 2015). Estonia’s wild bilberry is reported to have higher levels of polyphenols than the European cranberry, black currant, strawberry, American cranberry, or red currant (Ehala et al., 2005). Del Bó et al. (2013) suggested that intake of blueberries affects selected markers of oxidative stress and vascular function in males. Blueberries are rich in vitamins A, B, C, E, and PP, which enhance antioxidant activity (Prior et al., 1998).

Most of the published results about the chemical composition of the blueberry relate to its cultivated variety (Prior et al., 1998; Giovanelli and Buratti, 2009; Dragović-Uzelac et al., 2010; Gündüz et al., 2015; Saral et al., 2015). Milivojević et al. (2012) reported that the variability in the chemical fruit composition (sugars, organic acids, and phenolic compounds) and the correlation between the total phenolic content and antioxidant capacity correspond to the genetic differences between cultivated highbush blueberry and wild bilberry (V. myrtillus). Stevenson and Scalzo’s (2012) study showed a significant difference in the content of anthocyanins and total polyphenols among cultivars, which implies that there is considerable scope for breeding programs in order to generate new cultivars with a higher content of these compounds.

Although a lot of research has been done on the antioxidant activity and the quality of highbush blueberry around the world, little is known about the quality and polyphenols content of the cultivated blueberry cultivars and wild bilberry in Bosnia and Herzegovina. Nevertheless, numerous plantations of highbush blueberry, covering an area of 0.1 to 30 ha, have boosted the production of this fruit in this country with far more to be achieved.

The aim of the present study was to evaluate the physicochemical properties of the most commonly cultivated blueberry cultivars and wild bilberry growing in the same geographical area (central Bosnia, Bugojno locality). Additionally, the correlations between fruit weight and fruit composition (soluble solids content, total sugars, titratable acidity, pH, and total phenol) were determined. Knowledge about the composition of blueberry/bilberry from this country is scarce. The results of our study may lead in the future to production growth of blueberries, as well as their increased consumption in Bosnia and Herzegovina.

2. Materials and methods

2.1. Plant material

The research was carried out during 2015 and 2016 in the area of Bugojno in central Bosnia and Herzegovina (coordinates: 44°3′22″N and 17°26′59″E) on private plantations (highbush blueberry cultivars included ‘Earliblue’, ‘Bluegold’, ‘Bluecrop’, and ‘Goldtraube’ (Vaccinium corymbosum L.) and in natural populations for wild bilberry (Vaccinium myrtillus L.) in the immediate vicinity in the same geographic area at an altitude of 570 m (Mount Stožer near the city of Bugojno). Plants of wild bilberry were identified by Professor Emina Ademović (Department of Biology, Džemal Bijedic University of Mostar, Mostar, Bosnia and Herzegovina). No specific permissions were required for this location or activity. The study was carried out on private property with the owner’s permission.

The highbush blueberry cultivars listed above were planted in the experimental field in 2011 under ecological conditions. The experimental plots were laid out in a completely randomized block design with five replications per cultivar. In each replication, five plants were cultivated. A dropwise irrigation regime was applied.

Samples of cultivated highbush blueberry were collected at the first harvest, whereas bilberries were harvested at full ripening (one time). Fruit of highbush blueberry at full maturity (the number of days after full bloom was as follows: for ‘Earliblue’ in 2015 – 79, in 2016 – 73; for ‘Goldtraube’ in 2015 – 78, in 2016 – 76; for ‘Bluegold’ in 2015 – 78, in 2016 – 76; for ‘Bluecrop’ in 2015 – 81, in 2016 – 79) was picked manually from different plots (10 berries per replication, total 50 berries per cultivar). In 2015, we sampled, consecutively, ‘Bluegold’ (8 July), ‘Earliblue’ (10 July), ‘Bluecrop’ (14 July), and finally ‘Goldtraube’ (15 July). In 2016, we harvested samples of ‘Earliblue’ (1 July), ‘Goldtraube’ (8 July), ‘Bluegold’ (9 July), and ‘Bluecrop’ (10 July).

In the case of wild bilberries, they were collected during the fruit-ripening stage (blue-dark fruit) from ten bilberry plants with similar characteristics with respect to sun exposure, moisture conditions, etc. In 2015, wild bilberries were collected on 29 July and in 2016 on 25 July. Distances between individual plants were less than 15 m taking into account the clonal propagation of blueberries and their growing form, rhizomatous. It allowed the maintenance
of uniformity in the properties of individual plants in the sample. In total 50 berries were randomly collected from 10 plants.

2.2. Meteorological data
The monthly average temperature (Table 1) and precipitation (Table 2) data for the trial years were obtained from the Federal Hydro Meteorological Institute of Bosnia and Herzegovina (www.fhmzbih.gov.ba).

2.3. Characteristics of the fruit
In the case of fruit weight, we made an assumption that it was the weight of a total 50 individual berries per cultivar in respect to five replications, with ten berries in each replication. Fruit weights of each blueberry cultivar and wild bilberry were measured with a digital balance (Kern 440, Kern and Sohn, Germany) with 0.01 g sensitivity. The physical and chemical analysis was performed in the laboratory at the University of Sarajevo Faculty of Agriculture and Food Science.

2.4. The sampling procedure and preparation of the extract for chemical analysis
Berries for chemical tests were frozen in liquid nitrogen, packed in polyethylene bags, and stored at –20 °C (for about 10 days). Before the analysis, the berries were partially defrosted and homogenized for 2 min using a T-18 Ultra Turrax (IKA – Labortechnik, Staufen, Germany). The homogenized mass was filtered through Chromafil AO-45/25 polyamide filters (Macherey Nagel, Düren, Germany) and processed by further methods. The chemical composition of the berries was determined in three replications for each parameter (soluble solids content, total sugars, titratable acidity, and total phenol).

2.5. The soluble solids content
The soluble solids content was determined with a manual Abbe refractometer (Euromex, Holland) for each cultivar individually and corrected to the equivalent reading at 20 °C (AOAC, 1995) and the values were expressed in °Brix.

2.6. Total sugars content
The total sugars content was determined volumetrically according to the Luff Schoorl method (Egan et al., 1981).

2.7. Titratable acidity and pH
The determination of titratable acidity was performed by titration with 0.1 N NaOH with the phenolphthalein indicator until color change (pH 8.1), and the values are expressed as citric acid in g/100 g of fresh weight (FW) of fruit. pH was directly measured in freshly extracted berry juice with a pH-meter (Mettler Toledo, Greifensee, Switzerland) according to the guidelines of AOAC (1995).

2.8. Extraction of phenolic compounds
Phenolic compounds were extracted from partially dehydrated blueberry. Five grams of the sample were extracted with 20 mL of 70% (v/v) aqueous methanol solution. The mixture was extracted for 20 min in a nitrogen stream and filtered through Whatman No. 40 paper in a vacuum filtration system. The residue after filtration was extracted again in the same manner and filtrates were combined in 50 mL of distillate supplemented with 80% ethanol.

2.9. Total phenols content
The content of total phenols was determined according to the Folin–Ciocalteu spectrophotometric (UV-Vis 1700 spectrophotometer, Shimadzu, Kyoto, Japan) procedure, using gallic acid as a standard for the calibration curve (Singleton and Rossi, 1965). The results were read at 765 nm and expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh weight of the fruit (mg GAE/100 g FW).

2.10. Statistical analysis
Statistical data processing was performed by analysis of variance (ANOVA). The differences statistically significant were determined using the Tukey–Kramer test at P ≤ 0.05. Correlations between the weight and chemical properties were evaluated by Pearson's test.

Table 1. Monthly average temperatures (°C) in Bugojno, Bosnia and Herzegovina (2015–2016) (www.fhmzbih.gov.ba).

<table>
<thead>
<tr>
<th>Year</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>0.1</td>
<td>1.3</td>
<td>5.3</td>
<td>9.1</td>
<td>15.6</td>
<td>18.0</td>
<td>22.7</td>
<td>21.3</td>
<td>16.1</td>
<td>10.9</td>
<td>4.5</td>
<td>−1.3</td>
</tr>
<tr>
<td>2016</td>
<td>0.9</td>
<td>6.7</td>
<td>5.9</td>
<td>12.3</td>
<td>13.6</td>
<td>19.0</td>
<td>20.9</td>
<td>18.4</td>
<td>14.9</td>
<td>9.5</td>
<td>5.5</td>
<td>−1.5</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Year</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>90.1</td>
<td>43.9</td>
<td>55.1</td>
<td>47.7</td>
<td>60.0</td>
<td>72.6</td>
<td>67.6</td>
<td>51.3</td>
<td>91.2</td>
<td>166.6</td>
<td>62.5</td>
<td>0.2</td>
</tr>
<tr>
<td>2016</td>
<td>29.2</td>
<td>103.2</td>
<td>76.3</td>
<td>47.0</td>
<td>89.3</td>
<td>54.3</td>
<td>67.5</td>
<td>92.2</td>
<td>100.7</td>
<td>76.8</td>
<td>98.7</td>
<td>7.1</td>
</tr>
</tbody>
</table>
3. Results and discussion
In the present study we examined the chemical composition of 'Earliblue', 'Bluegold', 'Bluecrop', 'Goldtraube', and wild bilberry. It is important to underline that several parameters, not only the cultivar, but also growing conditions (weather, nutrition and soil properties, agronomic practices), harvesting, maturity stage, and transport and handling conditions influence the chemical composition of blueberry (Skupień, 2006; Stajčić et al., 2012; Rohloff et al., 2015; Gündüz et al., 2015; etc.).

3.1. Fruit weight
The results concerning the weight of cultivated blueberry and wild bilberry in 2015 and 2016 are presented in Figure 1 and Table 3. The average weight of the cultivated blueberry varied. 'Bluegold' (2.08–2.11 g) and 'Bluecrop' (2.07–2.11 g) had the highest, while 'Goldtraube' (1.12–1.22 g) had the lowest average weight. The highest impact of the year on fruit weight was observed in the case of 'Earliblue': in 2015, the average fruit weight was 1.49 g and in 2016 it was 1.90 g (an increase of 27%). Statistically significant differences were found between the cultivars for the fruit weight parameter. The highest differences were observed between 'Earliblue' and 'Goldtraube' in 2015 (difference 0.41 g) and between 'Bluegold' and 'Goldtraube' in 2016 (difference 0.78 g). The results are presented in Table 3.

Table 3. Physicochemical properties of highbush blueberry fruit and wild bilberry fruit (mean ± SD).

<table>
<thead>
<tr>
<th>Property</th>
<th>Year</th>
<th>Blueberry 'Earliblue'</th>
<th>Blueberry 'Bluegold'</th>
<th>Blueberry 'Bluecrop'</th>
<th>Blueberry 'Goldtraube'</th>
<th>Wild bilberry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit weight (g; N = 50 berries)</td>
<td>2015</td>
<td>1.49 ± 0.27</td>
<td>2.11 ± 0.58</td>
<td>2.07 ± 0.56</td>
<td>1.12 ± 0.20</td>
<td>0.28 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>1.90 ± 0.12</td>
<td>2.08 ± 0.51</td>
<td>2.11 ± 0.50</td>
<td>1.22 ± 0.27</td>
<td>0.32 ± 0.13</td>
</tr>
<tr>
<td>Soluble solids content (^Brix; N = 3 replications)</td>
<td>2015</td>
<td>13.0 ± 0.8</td>
<td>12.4 ± 0.2</td>
<td>13.3 ± 0.1</td>
<td>12.5 ± 0.3</td>
<td>11.1 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>13.2 ± 0.6</td>
<td>12.6 ± 0.2</td>
<td>13.7 ± 2.2</td>
<td>12.8 ± 2.2</td>
<td>11.3 ± 2.2</td>
</tr>
<tr>
<td>Total sugars (%) (N = 3)</td>
<td>2015</td>
<td>7.32 ± 0.42</td>
<td>7.30 ± 0.40</td>
<td>9.73 ± 0.18</td>
<td>8.76 ± 1.06</td>
<td>7.23 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>7.56 ± 0.51</td>
<td>7.62 ± 0.11</td>
<td>9.94 ± 0.03</td>
<td>8.93 ± 1.00</td>
<td>7.37 ± 0.91</td>
</tr>
<tr>
<td>Titratable acidity (g of citric acid/100 g FW; N = 3)</td>
<td>2015</td>
<td>0.84 ± 0.02</td>
<td>0.87 ± 0.02</td>
<td>0.70 ± 0.02</td>
<td>0.92 ± 0.03</td>
<td>0.99 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>0.86 ± 0.04</td>
<td>0.88 ± 0.02</td>
<td>0.72 ± 0.04</td>
<td>0.93 ± 0.03</td>
<td>1.02 ± 0.02</td>
</tr>
<tr>
<td>Total phenols (mg GAE/100 g FW; N = 3)</td>
<td>2015</td>
<td>288 ± 6</td>
<td>290 ± 3</td>
<td>264 ± 1</td>
<td>289 ± 8</td>
<td>431 ± 5</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>291 ± 7</td>
<td>294 ± 5</td>
<td>280 ± 5</td>
<td>309 ± 10</td>
<td>455 ± 5</td>
</tr>
<tr>
<td>pH (N = 3)</td>
<td>2015</td>
<td>3.5 ± 0.02</td>
<td>3.4 ± 0.01</td>
<td>3.5 ± 0.07</td>
<td>3.4 ± 0.02</td>
<td>3.3 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2016</td>
<td>3.6 ± 0.01</td>
<td>3.4 ± 0.03</td>
<td>3.4 ± 0.02</td>
<td>3.3 ± 0.05</td>
<td>3.2 ± 0.07</td>
</tr>
</tbody>
</table>
differences were observed between ‘Earliblue’ and all other cultivars (‘Bluegold’, ‘Bluecrop’, and ‘Goldtraube’), between ‘Bluegold’ and ‘Earliblue’ and ‘Goldtraube’, and between ‘Bluecrop’ and ‘Earlyblue’ and ‘Goldtraube’. There were no significant differences between ‘Bluegold’ and ‘Bluecrop’. It was also observed that variations in fruit weight were greater among genotypes than between growing seasons, which fits well with the results presented by Howard et al. (2003). Differences in fruit weight, besides genetic predisposition, were caused by different weather conditions during the years of research and most of all were pronounced in ‘Earliblue’ as an early cultivar. Temperature did not have a big influence on the fruit weight of ‘Bluegold’, ‘Bluecrop’, or ‘Goldtraube’ or on wild bilberry.

Similar results for average fruit weight of ‘Earliblue’ were also reported by Ścibisz and Mitek (2007) for blueberry from central Poland (1.4 g). Correia et al. (2016) found that Portugal’s ‘Bluecrop’ had a fruit weight of 1.93 g and ‘Goldtraube’ 1.36 g, which overlaps with our results. A similar average fruit weight of ‘Bluecrop’ (1.8 g) was also reported by Arsov et al. (2010) in Macedonia, by Milivojević et al. (2016) in Serbia (from 1.86 to 1.94 g), and by Zorenc et al. (2016) in Slovenia (from 1.82 to 1.83 g). Kim et al. (2013) recorded results similar to ours for ‘Bluecrop’ (1.83 g), ‘Bluegold’ (1.96 g), and ‘Earliblue’ (2.21 g) in Korea. A fresh fruit weight lower than ours was found by Leposavić (2014) for ‘Bluecrop’ in Čačak (Serbia), which was 1.58 g, and by Scibisz and Mitek (2007) for ‘Bluecrop’ (1.7 g) and ‘Bluegold’ (1.6 g) grown in central Poland and significantly lower for ‘Bluecrop’ (0.83 g) from Eastern Anatolia in Turkey (Çolak et al., 2017). If the average fruit weights are to be a criterion, ‘Bluegold’ and ‘Bluecrop’ cultivars can be recommended for further research in Bosnia and Herzegovina.

The results of average fruit weight of wild bilberry (0.28 g in 2015 and 0.32 g in 2016) in our research are similar to those recorded by Giovanelli and Buratti (2009): 0.28–0.29 g (Italy), Çolak et al. (2017): 0.23–0.34 g (Eastern Anatolia in Turkey), and Celik et al. (2018): 0.20–0.29 g (Çoruh valley in northeastern Turkey).

3.2. Soluble solids content

Soluble solids content is a measure of sweetness (Cortés-Rojas et al., 2016). The higher the content of total solids, the more convenient and desirable it is to process the fruit (Stajčić et al., 2012). The results of the analysis of soluble solids content (°Brix) in ‘Earliblue’, ‘Bluegold’, ‘Bluecrop’, ‘Goldtraube’, and wild bilberry are presented in Figure 2 and Table 3. The content of soluble solids in blueberry can increase with higher exposure to sunlight, which accelerates the photosynthetic rate (Cortés-Rojas et al., 2016). In our study ‘Bluecrop’ had the highest content of soluble solids in 2016 (13.7 °Brix), while in 2015 there was no significant difference between this cultivar and ‘Earliblue’ (13.3 and 13.0 °Brix, respectively). ‘Bluegold’ (from 12.4 to 12.6 °Brix) and ‘Goldtraube’ (from 12.5 to 12.8 °Brix) had similar values of soluble solids content in both years. Since the soluble solids content is a parameter that accounts for the quality of blueberry, the highest value was obtained for ‘Bluecrop’ and this cultivar can be recommended for further study.

Similar results of soluble solids content for ‘Bluecrop’ (from 10.4% to 12.8%) were also reported by Milivojević et

Figure 2. The average content of soluble solids in blueberry cultivars and wild bilberry (°Brix) ± SD (Remark: different letters at average values indicate that the cultivars differ significantly in the investigated property according to the Tukey–Kramer test with P ≤ 0.05). The same letters (e.g., a, a) – differences are not statistically significant, different letters (e.g., d, e) – the differences are statistically significant
The soluble solids content for 'Bluecrop' in our research was slightly higher than that reported by Leposavić (2014) in Serbia (11.1%) and by Arsov et al. (2010) in Macedonia (from 10.6% to 11.0%) and considerably higher than that recorded by Kim et al. (2013) in Korea (9.8 °Brix). The same was found for 'Bluegold' (9.8 °Brix) and 'Earliblue' (11.6 °Brix). Only Ribera et al. (2010) and Gündüz et al. (2015) recorded a higher amount of soluble solids in 'Bluegold' (13.7 and 13.2 °Brix) than in the present study.

In our research, the content of soluble solids in wild bilberry was 11.1 °Brix in 2015 and 11.3 °Brix in 2016. These results are comparable to those presented by Rohloff et al. (2015) for different locations of wild bilberry cultivations in Norway (from 8.24 to 11.1 °Brix) and to those obtained by Starast et al. (2007) for the wild bilberry in southern Estonia (10%). Çolak et al. (2017) reported a slightly higher content of soluble solids (11.5%–12.5%) in wild bilberry in Eastern Anatolia (Turkey) as compared to our research, while Stajčić et al. (2012) obtained a considerably higher content of soluble solids in bilberry in Serbia (Kopaonik) (14.7%) and Ochmian et al. (2009) in bilberry in Poland (13%).

3.3. Total sugars content
Total sugars content is a factor that defines fruit inner quality or the organoleptic properties (e.g., fruit flavor) (Stajčić et al., 2012; Li et al., 2013; Mikulic-Petkovsek et al., 2014; Okan et al., 2018). The accumulation of sugars in blueberry highly depends on the climate conditions (Correia et al., 2016). Their content in blueberry/bilberry is indirectly affected by light intensity because the metabolism of fruit depends on photosynthesis (Li et al., 2013; Mikulic-Petkovsek et al., 2014). The results of total sugars content in 'Earliblue', 'Bluegold', 'Bluecrop', 'Goldtraube', and wild bilberry for two consecutive years are presented in Figure 3 and Table 3.

'Bluecrop' had the highest content of total sugars in both years (9.73%–9.94%), whereas that for 'Goldtraube' was slightly lower (8.76%–8.93%). There were no significant differences between 'Earliblue' (7.32%–7.56%) and 'Bluegold' (7.30%–7.62%). The results obtained for 'Bluecrop' are comparable to those recorded by Leposavić (2014) in Serbia (8.95%) and by Zorenc et al. (2016) in Slovenia (6.19%–8.79%), but considerably lower than those presented by Milivojević et al. (2012) for blueberry in Serbia (15.6%) and by Skupień (2006) for the same in Poland (11.8%). Tested in the present study, the 'Bluecrop' cultivar, with the highest content of total sugars, is recommended for further experiments. The fact that 'Bluecrop' had the highest soluble solids content fits well with the results obtained in this section. The highest share in total solids of fruit is contributed by carbohydrates, i.e. sugars (Stajčić et al., 2012). The highest soluble solids content and total sugar content in 'Bluecrop' can also imply that it was more mature than the other cultivars at the same time of harvest.

The content of total sugars in wild bilberry ranged from 7.23% in 2015 to 7.37% in 2016. These values were much higher than the results presented by Mikulić-Petkovšek et al. (2016) in Serbia, by Skupień (2006) in Poland (13.3%), by Gündüz et al. (2015) in Michigan (USA) (12.9%), by Çolak et al. (2017) in Turkey (13.3%), and by Celik et al. (2018) in the Çoruh valley in northeastern Turkey (13.3%).
et al. (2014) for wild bilberry in Slovenia (3.81%–6.27% depending on the location), but less than for wild bilberry in Serbia: 7.84 ± 0.08% (Stajčić et al., 2012) and 15.3 ± 1.3% (Milivojević et al., 2012).

3.4. Titratable acidity
Titratable acidity has been shown to be significantly different among various cultivars (Skupień, 2006; Starast et al., 2007). Moreover, environmental and developmental factors can affect acidity levels in blueberry. This parameter can also influence other blueberry factors such as development of fruit color or decay susceptibility (Ehlenfeldt et al., 1994). The results of the 2-year research on the content of titratable acidity in 'Earliblue', 'Bluegold', 'Bluecrop', 'Goldtraube', and wild bilberry are presented in Figure 4 and Table 3. 'Bluecrop' had the lowest content of titratable acidity (0.70–0.72 g/100 g) and 'Goldtraube' had the highest one (0.92–0.93 g/100 g) among the cultivars examined. 'Earliblue' (0.84–0.86 g/100 g FW) and 'Bluegold' (0.87–0.88 g/100 g FW) had a similar content of titratable acidity. Comparable results for 'Bluecrop' were reported by Skupień (2006) in Poland (0.80%), by Kim et al. (2013) in Korea (0.78%), by Leposavić (2014) in Serbia (0.78%), and by Milivojević et al. (2016) in Serbia (0.57%–0.75%). Arsov et al. (2010) in Macedonia (1.17%) and Celik et al. (2018) in Turkey (1.3%) showed a significantly higher content of titratable acidity for this cultivar. According to Kim et al. (2013), 'Earliblue' had a considerably higher content of titratable acidity (0.72%) than it had our research.

Titratable acidity of wild bilberry ranged from 0.99 to 1.02 g/100 g and it was comparable to the results obtained by Giovanelli and Buratti (2009) in Italy (1.00%–1.18%) and Rohloff et al. (2015) in Norway (from 1.09% to 1.40%) and higher than that reported in the research conducted by Marjanović-Balaban et al. (2012) in the eastern part of Bosnia and Herzegovina (0.4%–0.7%) and Stajčić et al. (2012) in Serbia (0.52%). According to Ochmian et al. (2009) Poland’s wild bilberry had the content of titratable acidity of 1.44%, which is considerably higher than that observed in our research and higher than that for the wild bilberry in northeastern and western Anatolia, Turkey (1.58%) (Çolak et al., 2016).

Generally it was found among all cultivars that titratable acidity decreased by as much as the soluble solids content decreased. Titratable acidity and soluble solids content are paired to calculate SSC/TA ratio as a measure of sweetness. According to Beaudry (1992), the acceptable range of SSC/TA in blueberry should be between 14 and 33. Our study showed that only 'Earliblue', 'Bluegold', and 'Bluecrop' met this condition.

3.5. pH of fruit
The results of the research on the pH of 'Earliblue', 'Bluegold', 'Bluecrop', 'Goldtraube', and wild bilberry are presented in Figure 5 and Table 3. All cultivars and wild bilberry had a fairly uniform pH. 'Earliblue' had the highest average pH (3.5–3.6). The value of pH for wild bilberry was 3.2–3.3. According to Leposavić (2014), 'Bluecrop' had a pH similar to that in our research (3.0), while Celik et al. (2018) reported a slightly lower pH (pH 2.9) in Turkey. Kim et al. (2013) recorded the following pH values: for 'Bluecrop' it was pH 4.0 (which is slightly higher than what we measured), for 'Bluegold' it was pH 3.6 (which is close to our results), and for 'Earliblue' it was pH 2.8 (which is significantly lower than what we obtained). The pH of

![Figure 4. The average content of titratable acidity in blueberry cultivars and wild bilberry (g/100 g FW) ± SD (Remark: different letters at average values indicate that the cultivars differ significantly in the investigated property according to the Tukey–Kramer test with P ≤ 0.05). The same letters (e.g., a, a) – differences are not statistically significant](image-url)
wild bilberry in our research is comparable to the values presented by Rimpapa et al. (2007) for bilberry from other localities in Bosnia (Busovača, Fojnica, Konjic) (3.0–3.05) and by Marjanović-Balaban et al. (2012) for bilberry in the eastern part of Bosnia and Herzegovina (3.3–3.5). Starast et al. (2007) obtained a higher pH of wild bilberry (3.8) in South Estonia. According to Rohloff et al. (2015), the wild bilberry in Norway had a slightly lower pH (2.7–2.9) than in our research; the same is true of the wild bilberry in northeastern and western Anatolia, Turkey (2.7) (Çolak et al., 2016).

3.6. The total phenols content

This parameter is known as a marker of the antioxidant capacity of blueberry; phenolic compounds are known to inhibit free radicals and prevent deformation of DNA (Giovanelli and Buratti, 2009; Stajčić et al., 2012; Saral et al., 2015; Okan et al., 2018).

Therefore, the identification of blueberry’s antioxidant activity is crucial for evaluation of its impact on human health (Stajčić et al., 2012). The results of the present research on the content of total phenols in ‘Earliblue’, ‘Bluegold’, ‘Bluecrop’, ‘Goldtraube’, and wild bilberry are presented in Figure 6 and Table 3.

The results show that the content of total phenols in cultivated blueberry ranged from 264 mg GAE/100 g of FW in 2015 for ‘Bluecrop’ to 309 mg GAE/100 g in 2016 for ‘Goldtraube’, in accordance with the literature data (e.g., Okan et al., 2018). The differences in the content of polyphenols in blueberry can result from various biotic and abiotic factors (e.g., irradiation, temperature, and pathogenic infection) (Kalt et al., 2001; Howard et al., 2003). It was also found that genetics (different cultivars) plays a more significant role in influencing phenol content in blueberries than the growing season, which is in agreement with the results presented by Howard et al. (2003).

‘Goldtraube’ had the highest content of total phenols (289–309 mg GAE/100 g of FW). A similar result for ‘Goldtraube’ was reported by Giovanelli and Buratti (2009) in the agroecological conditions of Italy (251 mg GAE/100 g FW). According to the same author, ‘Bluecrop’ had content of total polyphenols equal to 299 mg GAE/100 g, which overlaps with our findings (264–280 mg GAE/100 g FW). Similar results for ‘Bluecrop’ were obtained by Dragović-Uzelac et al. (2010) in northern Croatia (292–368 mg GAE/100 g FW) and by Skupień (2006) in Poland (307 mg GAE/100 g FW). Several authors obtained slightly higher contents of total phenols for ‘Bluecrop’—for example, 327 mg GAE/100 g FW in the blueberry in Turkey (Celik et al., 2018), 417 mg GAE/100 g FW in the blueberry in central Turkey.
Poland (Ścibisz and Mitek, 2007), and 652 mg GAE/100 g FW in the blueberry in Romania (Bunea et al., 2011).

The results for total phenols in 'Bluecrop' in our research were significantly higher than those presented by Milivojević et al. (2012) for the blueberry in Serbia (199 mg GAE/100 g FW), by Okan et al. (2018) for the blueberry in northeastern Turkey (83.2–123.5 mg GAE/100 g FW), and by Kim et al. (2013) for the blueberry in Korea (205 mg GAE/100 g FW). 'Bluegold' and 'Earliblue' had similar contents of total phenols (288–294 mg GAE/100 g FW), which corresponds to the findings reported by Kim et al. (2013) for 'Bluegold' (290 mg GAE/100 g FW) and 'Earliblue' (267 mg GAE/100 g FW) in Korea. Okan et al. (2018) obtained a lower content of total phenols for cultivars in northeastern Turkey: 'Bluegold' had 106–164 mg GAE/100 g FW and 'Earliblue' 124 mg GAE/100 g FW.

The content of polyphenols in wild bilberry studied in the present research ranged from 431 to 455 mg GAE/100 g FW and it was similar to the results obtained by Milivojević et al. (2012) in Serbia (387 mg GAE/100 g FW) and by Jovančević et al. (2011) in 11 locations of different exposures, altitude, and habitat in Montenegro (392 to 524 mg GAE/100 g FW), but higher than the result recorded by Okan et al. (2018) for bilberry in northeastern Turkey (200–215 mg GAE/100 g FW). Rimpapa et al. (2007) observed a higher value of total phenol for bilberry in different locations in Bosnia (803–1040 mg/100 g FW), as well as Ochmian et al. (2009) for bilberry grown near Sulechów in west Poland: total phenol content was 640 mg/100 g. Rohloff et al. (2015) reported a higher content of total phenols (531–674 mg GAE/100 g FW) for the wild bilberry at different locations in Norway and Stajčić et al. (2012) in Serbia (808 mg GAE/100 g FW). Giovanelli and Buratti (2009) observed a similar value of the content of total polyphenols for the bilberry (577–614 mg GAE/100 g FW) in Italy, while Bunea et al. (2011) found a significantly higher content of total polyphenols in the wild bilberry in Romania (673–819 mg GAE/100 g FW). According to Može et al. (2011), pedoclimatic factors cause certain differences in the content of total phenols because there is no genetically induced variabiity.

3.7. Correlation analysis for blueberry

In the present study it was shown that the nutritional composition of blueberries varies with the season and cultivar, but also with growing location, when compared with other literature data (Häkkinen and Törrönen, 2010; Stajčić et al., 2012; Correia et al., 2016). We used Pearson’s correlation tests to determine the relation of fruit weight and chemical properties of blueberry (total polyphenol, total sugars, titratable acidity, soluble solids content, and pH). The results are shown in Table 4. It turned out that there was a high correlation between the fruit weight and total phenols, the content of total sugars, and soluble solids content, and a medium one between the content of titratable acidity and pH. In addition, there was a high correlation between the content of total phenols with the content of total sugar, solids content, and the content of titratable acidity, and a medium one with pH. While there was a strong positive correlation between the content of total sugar and the total acid and a medium correlation...
between total sugars and soluble solids content, there was a slight correlation between total sugar and pH. There was a medium correlation between titratable acidity and the soluble solids content and no correlation between titratable acidity and pH. There was a strong positive correlation between the soluble solids content and pH.

The results in our research showed variations between fruit of cultivated blueberry (‘Earliblue’, ‘Bluegold’, ‘Bluecrop’, and ‘Goldtraube’) and wild blueberry in physical (fruit weight) and chemical properties (soluble solids content, total sugars, titratable acidity, pH value of fruit, and total phenols). In our study, highbush blueberry and wild bilberry were cultivated under the same growing conditions and thus the genotypic effect seems to be dominant. Regarding the obtained results for physical (fruit weight) and chemical properties, all the cultivars are valuable and it is necessary to expand their production in Bosnia and Herzegovina to order to increase the consumption of this fruit. According to our findings, among the tested cultivated cultivars, ‘Bluecrop’ blueberry is the most suitable for the Bosnia and Herzegovina region, taking into account its weight and chemical composition (the highest values of soluble solids content and total sugars). A rich chemical composition of wild bilberry (especially the high content of polyphenols) can be a good stimulant for cultivated production in field conditions. Further studies should expand our knowledge about the chemical composition in fruit of cultivated cultivars and the native population in order to select the best ones for breeding strategies and production of a high value functional food. In addition, it is necessary to ensure the sustainable use of the potential of wild bilberry as a significant resource in natural populations. Further study is necessary, considering that aside from the environmental factors genetic predisposition has a significant influence on the physical and chemical composition of bilberry. Further genetic analyses of bilberry populations might shed additional light on the results obtained in the present study.

Table 4. Pearson’s correlation matrix for the fruit weight and the chemical properties of blueberries.

<table>
<thead>
<tr>
<th></th>
<th>Total phenols</th>
<th>Total sugars</th>
<th>Titratable acidity</th>
<th>Soluble solids content</th>
<th>pH</th>
<th>Fruit weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total phenols</strong></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total sugars</strong></td>
<td>–0.7867</td>
<td>–0.7213</td>
<td>0.8469*</td>
<td>0.6223</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Titratable acidity</strong></td>
<td>–0.5931</td>
<td>0.2290</td>
<td>0.8797*</td>
<td>0.6223</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Soluble solids content</strong></td>
<td>–0.9395*</td>
<td>–0.3546</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>–0.6788</td>
<td>–0.7213</td>
<td>0.5736</td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Correlation is significant at P ≤ 0.05 level (2-tailed)

References


Leposavić A (2014). Pomological properties of newly-produced cultivars of high-grade blueberry (Vaccinium corymbosum L.). PhD, Novi Sad, BIH.


