

## Seasonal Changes in Black Currant Fruit Quality

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### ABSTRACT

An experiment was conducted during eight years (2012–2019) to examine the effect of climatic variations (air temperature and precipitation) on the nutritional quality of berries of three black currant cultivars ('Ben Lomond', 'Titania' and 'Čačanska Crna'). HPLC was used for the determination of sugars and organic acids extracted from berries, while ascorbic acid and minerals were evaluated by spectrophotometry. Results indicated that the chemical profile of black currants varied among cultivars. 'Ben Lomond' and 'Čačanska Crna' exhibited excellent chemical characteristics of the berries, primarily in terms of their high sugars and organic acids content, but 'Čačanska Crna' stood out for its highest values of ascorbic acid. Also, seasonal variations caused by temperatures and precipitation affected the biosynthesis of primary metabolites in berries. The heavier precipitation and lower temperatures during berry formation and ripening promoted the accumulation of organic acids and ascorbic acid, as well as minerals P and Fe. Conversely, moderate temperatures and rainfall amounts promoted the synthesis of soluble solids, sugars and proteins, whereas higher temperatures and lower precipitation amount enhanced accumulation of the other tested minerals. PCA analysis showed the correlations among the cultivar/year interactions and identified group patterns. The results showed that the chemical properties of black currant not only depend on the genetic predisposition of the cultivar but also on climatic conditions like air temperature and precipitation, which have an important effect on nutrient metabolism in plants and promote biosynthesis and accumulation of primary metabolites in berries.

**Keywords:** Climatic variations, Primary metabolites, *Ribes nigrum* L.

### INTRODUCTION

According to the extent of worldwide production, currant has the second place among berry fruits, after strawberry. The present state of black currant (*Ribes nigrum* L.) production in Serbia is negligible, although there are demands for this fruit species and favorable natural conditions for its growing. The importance of currant growing is mainly based on its early cropping, high and regular yields and relatively less investments in establishment and maintenance of plantations. Black currant plants best thrive in humid mountainous areas

characterized by cool summers, high precipitation amounts and high humidity levels. It is characterized by winter hardiness, while high summer temperatures and drought during summer cause problems in black currant cultivation and directly affect productivity and fruit quality (Nikolić and Milivojević, 2010). The chemical composition of black currant changes significantly from year to year, demonstrating the influence of climatic conditions on overall fruit quality (Zheng *et al.*, 2009). Variations in climatic conditions have been documented to result in fruits with different chemical properties. Climatic conditions are one of the

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important factors that regulate the biosynthesis and accumulation of metabolites in berries (Watson *et al.*, 2002). Notably, temperatures and precipitation may have a negative or positive influence on the accumulation of primary metabolites in fruits depending on plant species/cultivars (Davik *et al.*, 2006; Poudel *et al.*, 2009; Uleberg *et al.*, 2012).

In general, berries of black currant are recognized as exceptionally rich sources of sugars and organic acids (Sturm *et al.*, 2003; Kafkas *et al.*, 2006). Changes in the content of sugars and acids can result in changes in fruit taste and flavor (Colaric *et al.*, 2005). Also, berries are an exhaustible source of ascorbic acid and minerals, especially potassium, calcium, magnesium, and iron (Anttonen *et al.*, 2006; Hegedűs *et al.*, 2008; Lefevre *et al.*, 2011). Sugars, acids, ascorbic acid and minerals are significant components contributing to the nutritional value and sensory properties of berries, which are very important in the biological processes of growth and development, as well as in preventing some chronic diseases (Gorinstein *et al.*, 2001). In recent years, climate change has shown warming tendencies, which can negatively affect the quality of black currant berries. Namely, climate change, especially changes in temperature and water availability, directly affects photosynthesis, often a modification of the chemical composition of the fruit, causing alterations in fruit quality (Moretti *et al.*, 2010; Shivashankara *et al.*, 2013; Stewart and Ahmed, 2020). Numerous studies have focused on the chemical properties of black currant berry, without examining its quality in response to climatic changes, temperature, and precipitation in different growing years.

Given the above, the purpose of this investigation was to assess berries quality properties of three black currant cultivars and explain how genotype and climatic conditions affect its variation over eight years.

## MATERIALS AND METHODS

### Plant Materials and Meteorological Data

The research was conducted at the Fruit Research Institute, Čačak, Western Serbia (43° 54' N latitude, 20° 21' E longitude, 242 m altitude), during eight years (2012–2019). Three black currant cultivars of different origins were used: 'Ben Lomond', 'Titania' and 'Čačanska Crna' (Table 1).

The experiment was set up in a randomized block design with three replications of each cultivar (90 bushes of total). During the trial, standard cultural practices (pruning, fertilisation and drip irrigation) were used.

Climate variables, including temperature (°C) and precipitation (mm) were recorded from May (berry formation) to June (berry ripening) during the experimental period (2012–2019). Data were obtained from the Republic Hydrometeorological Service of Serbia (Figure 1).

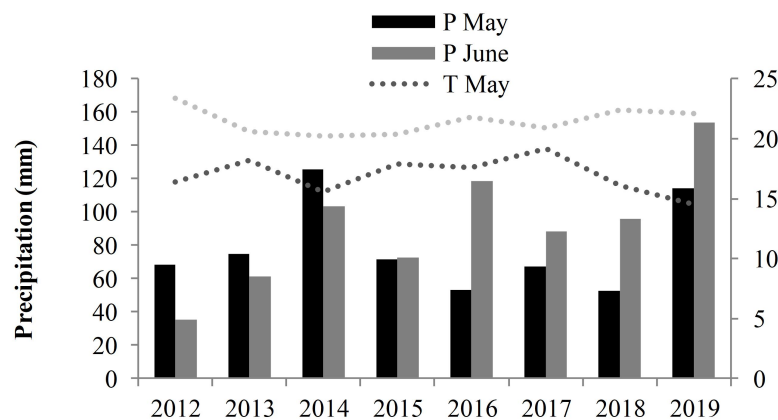
Black currant berries were sampled during June in full ripeness based on organoleptic properties. Berries were picked from the interior and exterior of each of the 30 bushes (100 g per bush) per cultivar. All samples from each replicate were pooled into a representative sample for further analysis.

### Fruit Quality Analysis

Approximately 100 g of fruit from each replicate was used to determine the content of primary metabolites. The soluble solids content was measured by a digital refractometer (Kruss, Germany). Total and invert sugars were assessed using the Loof-Schoorl method by Egan *et al.* (1981) and expressed as percentage. Total acids were assayed by neutralization to pH 7.0 with 0.1N NaOH, and expressed as percentage, while the pH value was measured by a pH Meter (Iskra MA 5707, Slovenia). Protein

**Table 1.** Origin, harvest time, source, and purpose of black currant cultivars.

| Cultivar        | Origin   | Harvest date                                       | Source   | Purpose   |
|-----------------|--|--|----------|---|
| ‘Titania’       | Altajskaja Desertnaja×<br>(Consort×Kajaanin Musta) | Mid-early<br>(during the second decade of<br>June) | Sweden   | Combined<br>traits<br>(Fresh<br>consumption,<br>freezing and<br>processing) |
| ‘Ben Lomond’    | (Brodtorp×Jaslunda) ×<br>(Consort×Magnus)          | Late<br>(during the third decade of June)          | Scotland |   |
| ‘Čačanska Crna’ | Open pollination of the cv.<br>‘Malling Jet’       | Mid-early<br>(during the second decade of<br>June) | Serbia   |   |



**Figure 1.** The average monthly temperatures and precipitation from May to June during eight years (2012–2019). P May: Precipitation in May; P June: Precipitation in June; T May: Temperature in May, T June: Temperature in June.

content was determined by Kjeldahl's method. Individual invert sugars were analyzed by HPLC (Thermo Scientific, Finnigan Spectra System, Waltham, MA, USA), as described by Jakopic *et al.* (2016). Organic acids were analyzed by the HPLC system, as described by Mikulic-Petkovsek *et al.* (2012). Sugars and organic acids were expressed as milligrams per gram of dry weight. Ascorbic acid content was measured by a Perkin Elmer UV/VIS spectrometer (Lambda 25), and absorbance was measured at 665 nm. Fifty microliters of a sample solution were mixed with 125  $\mu\text{L}$  of MB ( $c=0.4 \text{ mmol L}^{-1}$ ) solution and diluted to 10 mL with distilled water. A linear relationship was obtained between the decreasing absorbance intensity and the concentration

of AA in the concentration range of the analytes between 0.001 and 0.05  $\text{mol L}^{-1}$ . Results were expressed as milligrams per 100 grams of fresh weight ( $\text{mg } 100 \text{ g}^{-1} \text{ FW}$ ). Minerals of the berries were performed by flame atomic absorption spectrometry using a Varian Spectra AA 200 instrument equipped with a GTA 110 graphite furnace (Varian, USA). The sample (0.3–0.5 g) was weighed on a Denver Instruments analytical balance TB-2150 and coated with 8 mL of concentrated  $\text{HNO}_3$  and 1.5 mL of 30%  $\text{H}_2\text{O}_2$ . Digestion was carried out according to the following temperature program: 5 min–250W–180°C (temperature in the reference cell) or 65°C (the temperature at the surface of the cuvette measured sensor IC); 5 min–400W (same temperature



criterion); 5 min-500W (same temperature criterion). After the completion of digestion, contents were quantitatively transferred to a volumetric flask of 50 mL and amended to the line deionized water ASTM class I (0.067 uS). The content of phosphorus was performed by the spectrophotometric method (Paunović *et al.*, 2020). Results were expressed as milligrams per 100 grams of fresh weight (mg 100 g<sup>-1</sup> FW).

### Statistical Analysis

Experimental data were processed using the Analysis Of Variance (ANOVA, F test). Differences between means during the eight years of the experiment were compared by LSD test at  $P \leq 0.05$  significance levels. Principal Component Analysis (PCA) was used to determine the most important variables that explain the correlations among the cultivar/year interaction and to identify group patterns, using the Statistica 7.0 package (StatSoft, Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

Primary metabolites in berries are genetically determined, but they can be affected by environmental conditions (e.g. light, photoperiod, temperature, etc.) and genotype×environment interactions (Tiwari and Cummins, 2013). The present results confirm the importance of genotype as the main factor that influenced the concentration of primary metabolites in black currant berries. However, the study of climatic conditions in currant cultivation is also important to understand the differences that occur in berries quality. Black currant berries have long been recognized as an excellent source of sugars and acids, as well as one of the important sources of ascorbic acid (Tables 2 and 3). Fructose and glucose are the major sugars present in large amounts compared with sucrose. With regard to acids, citric acid is the most abundant acid, while malic acid is present in

minor concentrations. The content of sugars considerably varied among cultivars. 'Čačanska Crna' had the highest content of total and invert sugars, as well as the highest content of fructose, and correspondingly showed the lowest amount of acids in berries. Compared to the other two cultivars, the changes were an average of 18.6% for total sugars, 12.3% for invert sugars and 11.5% for fructose. Also, 'Čačanska Crna' was the cultivar with the highest content of proteins and ascorbic acid, whose detected concentrations were higher by an average of 10.3 and 7.22%, respectively, than the cultivars 'Ben Lomond' and 'Titania'. In contrast, 'Ben Lomond' exhibited the highest content of soluble solids, glucose and sucrose, and relatively higher levels of all tested acids. In the berries of this cultivar, the average content of glucose and sucrose was 12.6 and 29.1%, respectively, higher than the other investigated cultivars, as well as the average content of citric (8.93%) and malic acid (18.6%). These data are comparable with the results of Milivojević *et al.* (2009) and Raudsepp *et al.* (2010).

With regard to minerals, the study demonstrated that black currants berries are an inexhaustible source of minerals, which make the berries highly valuable (Tables 4 and 5). Analyses of black currant revealed that the berries contained a high level of macro-elements, especially K, followed by P, Ca, Mg and Na, and, to a lesser amount of micro-elements (Cu, Zn and Fe). 'Čačanska Crna' contained, on average, the highest amounts of P, Na and Ca, whereas 'Ben Lomond' registered higher levels of K, Mg, and micro-elements.

With respect to years, the data in Tables 2 and 3 reveal that the content of sugars and acids changed during the examination years. The heavier rainfall (392.7 mm) and lower air temperature (19.3°C) in 2014 stimulated the biosynthesis of acids, especially citric and malic acids, as well as minerals P and Fe. The value of citric acid in the berries was 2.65 times higher than malic acid. When comparing years, the level of citric and malic acid in 2014 increased by an average

**Table 2.** Basic chemical properties of black currant berries during an eight-year period. <sup>a</sup>

| Cultivar/<br>Year | Soluble<br>solids<br>(%) | Total<br>sugars<br>(%) | Invert<br>sugars<br>(%) | pH           | Total<br>acids<br>(%) | Proteins<br>(%) |
|-------------------|--------------------------|------------------------|-------------------------|--------------|-----------------------|-----------------|
| ‘Ben Lomond’      | 16.01±0.29 a             | 9.35±0.28 c            | 7.60±0.14 c             | 3.26±0.09 a  | 2.73±0.11 a           | 1.09±0.04 b     |
| ‘Titania’         | 15.99±0.26 a             | 12.45±0.42 b           | 8.57±0.20 b             | 3.14±0.07 b  | 2.58±0.08 b           | 1.08±0.03 b     |
| ‘Čačanska Crna’   | 15.43±0.16 b             | 13.40±0.43 a           | 9.22±0.28 a             | 3.04±0.04 c  | 2.61±0.09 b           | 1.21±0.06 a     |
| 2012              | 15.31±0.15 c             | 10.73±0.67 d           | 8.11±0.54 cd            | 3.29±0.04 b  | 1.52±0.02 f           | 1.39±0.04 a     |
| 2013              | 16.97±0.37 a             | 12.40±0.74 c           | 8.64±0.69 b             | 3.16±0.03 cd | 2.83±0.05 c           | 1.17±0.01 d     |
| 2014              | 14.39±0.09 d             | 10.10±0.56 e           | 7.82±0.40 e             | 3.08±0.03 e  | 3.10±0.09 a           | 1.09±0.01 e     |
| 2015              | 14.93±0.11 c             | 12.56±0.76 c           | 8.49±0.61 bc            | 2.57±0.01 f  | 2.72±0.04 d           | 1.25±0.03 b     |
| 2016              | 16.83±0.23 a             | 14.91±0.88 a           | 9.47±0.89 a             | 3.60±0.06 a  | 2.96±0.07 b           | 1.40±0.05 a     |
| 2017              | 16.18±0.33 b             | 13.08±0.82 b           | 8.85±0.85 ab            | 3.15±0.03 cd | 2.54±0.03 e           | 0.80±0.01 f     |
| 2018              | 16.89±0.43 a             | 10.54±0.63 d           | 8.36±0.56 bc            | 3.19±0.03 c  | 2.58±0.04 e           | 0.71±0.01 g     |
| 2019              | 14.99±0.13 c             | 9.55±0.47 f            | 7.98±0.43 e             | 3.11±0.02 de | 2.87±0.06 c           | 1.22±0.02 c     |
| ANOVA             |                          |                        |                         |              |                       |                 |
| Cultivar (A)      | **                       | **                     | **                      | **           | **                    | **              |
| Year (B)          | **                       | **                     | **                      | **           | **                    | **              |
| A×B               | **                       | **                     | **                      | **           | **                    | **              |

<sup>a</sup> (a-g) Mean values followed by a different lower-case letter in each column were significantly different at  $P \leq 0.05$  (LSD test).

**Table 3.** Content of individual invert sugars, organic acids and ascorbic acid in black currant berries during an eight-year period. <sup>a</sup>

| Cultivar/<br>Year | Fructose<br>(mg g <sup>-1</sup> ) | Glucose<br>(mg g <sup>-1</sup> ) | Sucrose<br>(mg g <sup>-1</sup> ) | Citric acid<br>(mg g <sup>-1</sup> ) | Malic acid<br>(mg g <sup>-1</sup> ) | Ascorbic acid<br>(mg 100 g <sup>-1</sup> ) |
|-------------------|-----------------------------------|----------------------------------|----------------------------------|--------------------------------------|-------------------------------------|--|
| ‘Ben Lomond’      | 127.06±2.01 b                     | 95.13±2.42 a                     | 19.35±1.41 a                     | 9.45±1.38 a                          | 3.83±0.33 a                         | 207.64±3.49 b                              |
| ‘Titania’         | 123.89±1.64 c                     | 82.87±1.59 b                     | 13.09±1.17 c                     | 8.81±1.32 b                          | 2.98±0.27 b                         | 201.95±3.20 c                              |
| ‘Čačanska Crna’   | 141.90±2.38 a                     | 83.35±1.97 b                     | 14.33±1.27 b                     | 8.40±1.19 c                          | 3.25±0.30 b                         | 220.73±4.09 a                              |
| 2012              | 118.70±1.78 d                     | 72.50±3.14 f                     | 7.28±1.03 e                      | 2.23±0.31 g                          | 1.34±0.13 e                         | 193.44±1.94 f                              |
| 2013              | 125.72±2.31 c                     | 76.87±2.62 e                     | 9.94±1.06 d                      | 12.78±1.02 c                         | 3.26±0.37 bc                        | 211.43±2.08 d                              |
| 2014              | 139.43±3.92 b                     | 84.19±3.28 d                     | 15.89±1.95 c                     | 19.84±1.25 a                         | 6.12±0.54 a                         | 220.12±2.50 b                              |
| 2015              | 147.62±4.18 a                     | 99.41±3.54 a                     | 27.15±2.96 a                     | 14.09±1.10 b                         | 3.61±0.34 b                         | 218.33±2.37 bc                             |
| 2016              | 128.53±2.28 c                     | 94.72±3.51 b                     | 10.32±1.49 d                     | 2.43±0.36 g                          | 1.74±0.21 d                         | 215.19±2.33 cd                             |
| 2017              | 129.27±2.47 c                     | 88.58±3.06 c                     | 18.79±2.49 b                     | 5.17±0.51 f                          | 3.43±0.43 b                         | 203.15±2.04 e                              |
| 2018              | 121.80±2.96 d                     | 90.31±3.26 c                     | 19.28±2.55 b                     | 6.32±0.74 e                          | 3.55±0.39 b                         | 183.74±1.63 g                              |
| 2019              | 132.16±3.23 c                     | 89.67±3.08 c                     | 16.07±2.03 c                     | 8.25±0.94 d                          | 2.93±0.24 c                         | 235.49±2.61 a                              |
| ANOVA             |                                   |                                  |                                  |                                      |                                     |  |
| Cultivar (A)      | **                                | **                               | **                               | **                                   | **                                  | **   |
| Year (B)          | **                                | **                               | **                               | **                                   | **                                  | **   |
| A×B               | **                                | **                               | **                               | **                                   | **                                  | **   |

<sup>a</sup> (a-g) Mean values followed by a different lower-case letter in each column were significantly different at  $P \leq 0.05$  (LSD test).

of 4.20 times and 2.46 times, respectively, and the content of P and Fe by an average of 1.10-fold and 1.96-fold, respectively, compared to the other experimental years.

Slightly lower rainfall amounts (322.4 mm) and higher temperatures (19.6 °C) in 2019 had a positive effect on the content of ascorbic acid (235.47 mg.100 g<sup>-1</sup>). Generally, ascorbic acid accumulation in the

berries was 1.14-fold higher compared to the other years.

The data are in agreement with the reports of Rubinskiene *et al.* (2006) and Vagiri *et al.* (2013), who found that higher amounts of rainfall and lower air temperatures during the growing season favor the synthesis of larger amounts of acids and ascorbic acid in the fruit. According to Lister *et al.* (2002),



black currants grown in the north have higher levels of ascorbic acids than those grown in the south. Conversely, Walker *et al.* (2010) detected that black currant growing on south-facing slopes contained 20% more ascorbic acid than black currant grown on north-facing slopes. Besides, Zheng *et al.* (2009) and Kaldmae *et al.* (2013) noted that temperature is negatively correlated with ascorbic acid content, as opposed to rainfall. Similarly, Sim *et al.* (2017) recorded a positive correlation between rainfall and the content of malic acid in chokeberry and blueberry.

Compared to 2014 and 2019, in 2016 air temperature increased by 1.6°C and precipitation amount decreased by 81.1 mm, which contributed to the increased metabolic accumulation of total sugars by 1.39-fold and invert sugars by 1.14-fold. It is assumed that higher temperature and more moderate rainfall directly increased the level of total and invert sugars, and decreased the concentration of acids in 2016. Also, the level of proteins was higher by 1.35-fold, as well as pH value by 1.17-fold than in the other tested years. The soluble solids content was relatively stable during the experimental years, given that their high value was recorded in 2013, 2016, and 2018. These years were characterized by higher average annual air temperatures (19.4, 19.7, and 21.0°C, respectively), and lower total precipitation (136.2, 171.6, and 148.5 mm, respectively), which is in agreement with the findings of Brennan (1996), who reported that warm weather conditions increase the soluble solids content and accumulate less acids in the fruits.

The synthesis of individual invert sugars content was the highest in 2015, which had more moderate air temperatures (19.1°C) and rainfall amounts (144.0 mm). Fructose, glucose and sucrose were identified in black currant berries and concentrations ranged between 118.70–147.62, 72.50–99.41, and 7.28–27.15 mg g<sup>-1</sup>, respectively. The fructose content was 1.50 times higher than glucose and 8.39 times higher than sucrose, which is comparable with the research by

Boccorh *et al.* (1998), who reported that the ratio of fructose/glucose in black currant berries differed from 1.2 to 1.6 depending on the year. The proportion of individual sugars also varied with year. Berries harvested in 2015 contained 1.15-fold fructose, 1.17-fold glucose and 2.19-fold sucrose concentration compared to the other experimental years. Sucrose was more variable during the study compared to fructose and glucose. These results can be supported by the findings of Rubinskiene *et al.* (2006) and Kaldmae *et al.* (2013), who reported a positive correlation between air temperature and the content of soluble solids and sugars, and a negative correlation between rainfall and these parameters. Similarly, Zheng *et al.* (2009) and Vagiri *et al.* (2013) discovered that elevated temperatures promote the accumulation of soluble solids and invert sugars in black currants. It is important to note that the concentration of these compounds was higher in currant fruits cultivated in the southern regions as compared to those grown in the northern regions. Contrary, Woznicki *et al.* (2017) found a decrease in fructose and glucose content in black currant berries with increasing temperature.

Extremely high average temperatures (23.4°C) and uncharacteristic drought (35.4 mm total rainfall) during berry ripening in 2012, favored accumulation of minerals K, Na, Ca, Mg, Cu and Zn in the berries, causing their increase compared to the other experimental years (Tables 4 and 5). The main macroelement in black currants berry was K, and the microelement Fe, confirming the previously established finding by Perkins-Veazie and Collins (2001), Hegedűs *et al.* (2008) and Lefevre *et al.* (2011). The level of K ranged from 305.79 mg 100 g<sup>-1</sup> (2016) to 352.78 mg 100 g<sup>-1</sup> (2012) and was 1.09 times higher than in other years. The content of all examined minerals significantly varied during the study period. The level of macro- (P, Na, Ca, Mg) and microelements (Cu and Zn) was higher in 2012, probably due to the increased synthesis of minerals in berries as a result of

**Table 4.** Content of macro-elements in black currant berries during an eight-year period. <sup>a</sup>

| Cultivar/<br>Year | K<br>(mg 100 g <sup>-1</sup> ) | P<br>(mg 100 g <sup>-1</sup> ) | Na<br>(mg 100 g <sup>-1</sup> ) | Ca<br>(mg 100 g <sup>-1</sup> ) | Mg<br>(mg 100 g <sup>-1</sup> ) |
|-------------------|--------------------------------|--------------------------------|---------------------------------|---------------------------------|---------------------------------|
| 'Ben Lomond'      | 338.31±3.13 a                  | 85.69±3.03 c                   | 22.85±0.73 c                    | 27.91±0.49 c                    | 28.40±1.01 a                    |
| 'Titania'         | 316.82±2.61 c                  | 87.75±3.57 b                   | 24.39±0.76 b                    | 30.06±0.57 b                    | 25.31±0.96 b                    |
| 'Čačanska Crna'   | 325.04±3.07 b                  | 96.42±4.37 a                   | 29.87±0.95 a                    | 35.18±0.61 a                    | 22.69±0.93 c                    |
| 2012              | 352.78±2.77 a                  | 79.70±1.27 f                   | 33.19±0.90 a                    | 35.55±0.55 a                    | 30.78±0.54 a                    |
| 2013              | 327.76±1.33 e                  | 89.34±1.50 d                   | 23.12±0.57 e                    | 28.01±0.23 f                    | 24.95±0.23 e                    |
| 2014              | 307.25±1.54 f                  | 97.96±2.63 a                   | 19.62±0.40 f                    | 31.88±0.37 c                    | 27.16±0.37 c                    |
| 2015              | 328.18±1.76 de                 | 93.13±2.50 b                   | 24.68±0.45 d                    | 29.23±0.28 e                    | 14.02±0.13 f                    |
| 2016              | 305.79±1.41 g                  | 90.15±2.19 d                   | 24.89±0.25 d                    | 30.70±0.34 d                    | 28.37±0.43 b                    |
| 2017              | 330.37±2.06 c                  | 88.34±1.49 e                   | 26.88±0.63 c                    | 31.01±0.38 cd                   | 26.94±0.33 cd                   |
| 2018              | 329.33±1.98 cd                 | 89.67±1.54 d                   | 28.57±0.81 b                    | 33.23±0.50 b                    | 26.53±0.32 d                    |
| 2019              | 332.29±2.34 b                  | 91.31±2.35 c                   | 24.63±0.37 d                    | 28.39±0.26 f                    | 25.04±0.24 e                    |
| ANOVA             |                                |                                |                                 |                                 |                                 |
| Cultivar (A)      | **                             | **                             | **                              | **                              | **                              |
| Year (B)          | **                             | **                             | **                              | **                              | **                              |
| A×B               | **                             | **                             | **                              | **                              | **                              |

<sup>a</sup> (a-g) Mean values followed by a different lower-case letter in each column were significantly different at  $P \leq 0.05$  (LSD test).

**Table 5.** Contents of microelements in black currant berries during an eight-year period. <sup>a</sup>

| Cultivar/<br>Year | Cu<br>(mg 100 g <sup>-1</sup> ) | Zn<br>(mg 100 g <sup>-1</sup> ) | Fe<br>(mg 100 g <sup>-1</sup> ) |
|-------------------|---------------------------------|---------------------------------|---------------------------------|
| 'Ben Lomond'      | 0.536±0.07 a                    | 0.341±0.05 a                    | 5.31±0.33 c                     |
| 'Titania'         | 0.472±0.11 c                    | 0.326±0.04 b                    | 6.09±0.39 a                     |
| 'Čačanska Crna'   | 0.517±0.08 b                    | 0.308±0.06 c                    | 5.67±0.36 b                     |
| 2012              | 0.681±0.07 a                    | 0.373±0.07 a                    | 3.64±0.08 e 5.03±0.14 d         |
| 2013              | 0.464±0.03 d                    | 0.279±0.02 c                    |                                 |
| 2014              | 0.508±0.04 c                    | 0.365±0.07 a                    | 9.77±0.28 a 4.95±0.11 d         |
| 2015              | 0.415±0.02 e                    | 0.261±0.04 c                    |                                 |
| 2016              | 0.587±0.06 b                    | 0.370±0.07 a                    | 6.28±0.22 b 5.16±0.16 cd        |
| 2017              | 0.513±0.05 c                    | 0.308±0.05 b                    |                                 |
| 2018              | 0.501±0.03 c                    | 0.325±0.06 b                    | 5.64±0.18 c 5.02±0.12 d         |
| 2019              | 0.398±0.01 e                    | 0.317±0.05 b                    |                                 |
| ANOVA             |                                 |                                 |                                 |
| Cultivar (A)      | **                              | **                              | **                              |
| Year (B)          | **                              | **                              | **                              |
| A×B               | **                              | **                              | **                              |

<sup>a</sup> (a-g) Mean values followed by a different lower-case letter in each column were significantly different at  $P \leq 0.05$  (LSD test).

higher temperatures during harvest season. The results are in agreement with the findings of Woolf *et al.* (1999), who found that avocado fruits had higher mineral accumulation (Ca, Mg and P) at a higher temperature, which may be related to water flow throughout the fruit. According to Stewart and Ahmed (2020), changes in temperature and precipitation affect the content of micronutrients in fruits based on geographical area. Contrary to our results,

Shivashankara *et al.* (2013) observed that higher temperatures and deficit in irrigation decrease the mineral content in fruit.

Principal Component Analysis (PCA) was used to the complete data set (basic chemical properties, primary metabolites, content of macro and microelements) to determine the most important variables that explained the correlations among cultivar/year combinations and to identify group patterns (Table 6). The five main components

**Table 6.** Loadings of the variables in PCA.

| Variable       | PC1     | PC2     | PC3    | PC4    | PC5    |
|----------------|---------|---------|--------|--------|--------|
| Soluble solids | 0.181   | 0.470   | -0.311 | -0.445 | 0.452  |
| Total sugars   | 0.290   | 0.419   | 0.506  | 0.527  | -0.013 |
| Invert sugars  | 0.422   | 0.211   | 0.572  | 0.260  | 0.396  |
| Total acids    | 0.899*  | -0.012  | -0.281 | -0.044 | -0.067 |
| pH             | 0.181   | 0.582   | -0.129 | -0.672 | -0.270 |
| Proteins       | -0.342  | 0.625   | 0.192  | 0.200  | 0.444  |
| Fructose       | 0.822*  | -0.134  | 0.360  | -0.101 | 0.039  |
| Glucose        | 0.478   | -0.390  | 0.665  | -0.052 | 0.365  |
| Sucrose        | 0.439   | 0.642   | -0.014 | -0.042 | 0.556  |
| Citric acid    | 0.616   | 0.077   | 0.490  | 0.053  | -0.119 |
| Malic acid     | 0.674   | -0.043  | 0.400  | -0.494 | 0.146  |
| Ascorbic acid  | 0.582   | -0.289  | 0.055  | 0.142  | -0.506 |
| K              | -0.648  | 0.641   | 0.230  | -0.128 | -0.002 |
| P              | 0.790*  | 0.230   | -0.227 | 0.231  | 0.081  |
| Na             | -0.863* | 0.322   | 0.078  | -0.202 | -0.016 |
| Ca             | -0.746* | -0.249  | 0.138  | 0.042  | 0.382  |
| Mg             | -0.391  | -0.561  | 0.538  | -0.367 | -0.005 |
| Fe             | 0.731*  | 0.172   | 0.307  | -0.486 | 0.024  |
| Zn             | -0.365  | -0.700* | 0.190  | -0.004 | -0.057 |
| Cu             | -0.637  | -0.435  | 0.302  | -0.306 | -0.010 |
| Eigenvalue     | 7.093   | 3.500   | 2.518  | 1.897  | 1.529  |
| Variance (%)   | 35.47   | 17.50   | 12.59  | 9.48   | 7.64   |
| Cumulative (%) | 35.47   | 52.97   | 65.56  | 75.05  | 82.69  |

\* Loadings higher than 0.70.

accounted for 35.47, 17.50, 12.59, 9.48, and 7.64% of the total variations among black currant cultivars based on 20 chemical traits during the eight-year period. The first PC-score was mainly dominated by total acids, fructose and minerals P and Fe in the positive direction, and minerals Na and Ca in the negative direction. Also, PC1 presented high loading, over 0.60 of absolute value, for citric and malic acids at the positive site, and minerals K and Cu at the negative site. The second PC-score (over 0.60 of absolute value) performed to high contribution for proteins, sucrose and mineral K in the positive direction, and mineral Zn in the negative direction. PC3 was positively correlated with glucose, while PC4 was negatively correlated with pH value. Scatterplot of chemical properties in three black currant cultivars over an eight-year is presented in Figure 2. PCA analysis demonstrated strong relations between the year and fruit quality. Years were well discriminated forming three separate

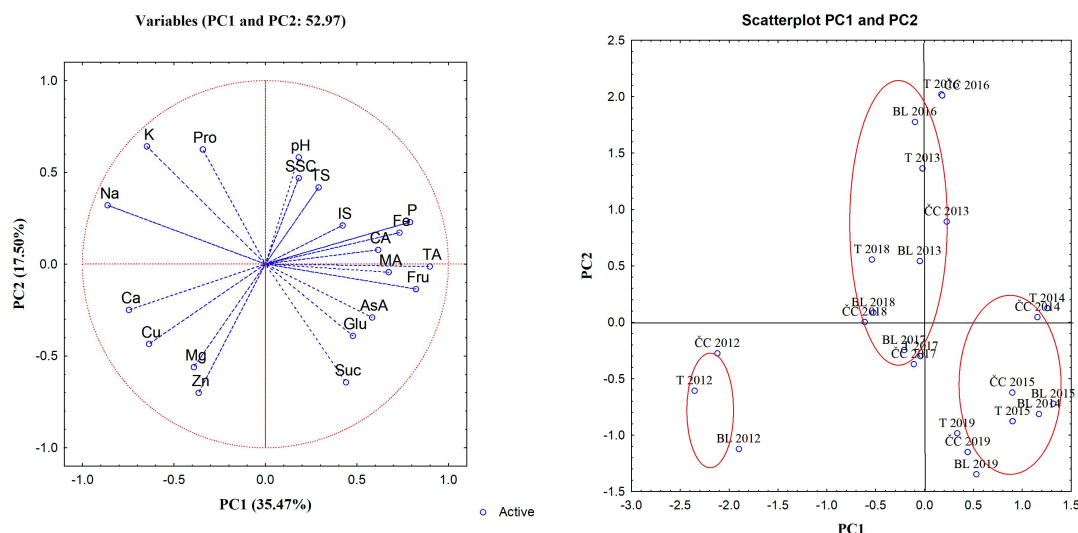
clusters. The first group, composed of all three cultivars in 2014, 2015, and 2019, was represented in the positive part of PC1, with a high level of individual invert sugars, total acids, organic acids, ascorbic acids and minerals P and Fe.

Higher levels of soluble solids, total and invert sugars, proteins and pH values were recorded in 2013, 2016, and 2018. Samples from the third group showed the lowest scores in 2012 for all tested parameters, except for the content of minerals that had the highest values.

## CONCLUSIONS

The results of the present research revealed that seasonal changes in temperature and precipitation during berry formation and ripening have an important effect on plant metabolism, and promote the synthesis of different primary metabolites in the berries, thus positively affecting the





**Figure 2.** Scatterplot for chemical analyses in black currant cultivars (A). Abbreviations used: TS- Total Sugars; IS- Invert Sugars; TA- Total Acids; Fru- Fructose; Glu- Glucose; Suc- Sucrose; CA- Citric Acid; MA- Malic Acid; AsA- Ascorbic Acid; pH- Fruit pH; Pro- Proteins; Minerals- K, P, Na, Ca, Mg, Fe, Zn and Cu. (B) Principal Component Analysis (PCA) received by analysis of three black currant cultivars during eight experimental years.

quality and commercial value of the fruit. PCA confirmed the existence of a positive correlation between most of the examined parameters of berry quality and low temperatures with higher rainfall amounts, as well as a negative correlation between the mentioned parameters and a high average temperature followed by drought.

This study also showed wide variations in the chemical properties of the cultivars examined, which clearly indicates that the differences in cultivars play an important role in determining fruit composition and give a unique perspective for further work on breeding programs. The tested cultivars 'Ben Lomond' and 'Čačanska Crna' can be recommended for widely growing in similar environmental conditions due to the established good quality of the fruit. In general, the results may assist in the selection of cultivars as well as in the selection of climatic conditions (i.e. temperature and precipitation during the berry-ripening period), leading to fruits with

enhanced taste and potential beneficial health-related properties.

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## تغییرات فصلی در کیفیت میوه کشمش سیاه (Black Currant)

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### چکیده

برای بررسی تأثیر تغییرات آب و هوایی (دمای هوا و بارش) بر کیفیت غذایی میوه‌های سه رقم کشمش سیاه به نامهای (Ben Lomond)، (Titania) و (Čačanska Crna)، آزمایشی در طول هشت سال (۲۰۱۲-۲۰۱۹) انجام شد. برای تعیین قندها و اسیدهای آلی استخراج شده از کشمش ها HPLC استفاده شد، در حالی که اسید اسکوربیک و مواد معدنی به وسیله اسپکتروفتومتری ارزیابی شد. نتایج نشان داد که مشخصات شیمیایی کشمش سیاه در بین ارقام متفاوت است. "Ben Lomond" و "Čačanska Crna" ویژگی های شیمیایی عالی کشمش ها را به نمایش گذاشتند، به ویژه از نظر محتوای قند و زیاد بودن اسیدهای آلی، اما "Čačanska Crna" به دلیل بالاترین مقادیر اسید اسکوربیک خود برجسته بود. همچنین، تغییرات فصلی ناشی از دما و بارش بر بیوسنتز متابولیت‌های اولیه در کشمش‌ها تأثیر گذاشت. بارش بیشتر و دماهای پایین تر در طول تشکیل و رسیدن کشمش‌ها باعث تجمع اسیدهای آلی و اسید اسکوربیک و همچنین مواد معدنی P و Fe شد. برعکس، دماهای متوسط و مقادیر بارندگی باعث افزایش سنتز مواد جامد محلول، قندها و پروتئین‌ها می‌شوند، در حالی که دماهای بالاتر و میزان بارندگی کمتر تجمع مواد معدنی دیگر را افزایش می‌دهند. تجزیه و تحلیل PCA همبستگی بین برهمکنش‌های رقم/سال و الگوهای گروهی را نشان داد. بر پایه نتایج، خواص شیمیایی کشمش سیاه نه تنها به استعداد ژنتیکی رقم بستگی دارد، بلکه به شرایط اقلیمی مانند دمای هوا و بارش نیز وابسته است و تأثیر مهمی بر متابولیسم عناصر غذایی در گیاهان دارد و باعث بیوسنتز و تجمع متابولیت‌های اولیه در کشمش‌ها می‌شود.