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# PRIMARY METABOLITES, VITAMINS AND MINERALS IN BERRY AND LEAF EXTRACTS OF BLACK CURRANT (*RIBES NIGRUM* L.) UNDER DIFFERENT SOIL MANAGEMENT SYSTEMS

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**Abstract**

This study examines the effect of soil management system (bare fallow, sawdust mulch and black plastic mulch) on the contents of primary metabolites [individual invert sugars (glucose, fructose, sucrose), organic acids (citric acid, malic acid), vitamins (C, A, B3) and minerals (K, P, Ca, Fe)] in berry and leaf extracts of seven black currant cultivars ('Ben Lomond', 'Ben Sarek', 'Tsema', 'Titania', 'Ćaćanska Crna', 'Tisel' and 'Tiben'). High performance liquid chromatography (HPLC) was employed for the identification of individual invert sugars, organic acids and vitamins (A, B3) extracted from berries and leaves. Vitamin C was evaluated by spectrophotometry, and minerals were determined by flame atomic absorption spectrophotometry. Soil management systems and cultivars showed highly significant differences in the levels of the tested parameters. Soil management system had a significant positive effect on the synthesis of primary metabolites, vitamins and minerals in both berries and leaves. The highest values in berry and leaf extracts were obtained by currants grown under sawdust and black plastic mulch, and the lowest by those on bare fallow. Also, the extracts exhibited different characteristics. Berries had a higher content of the tested parameters than leaves. The results indicated that black currant berries and leaves show good adaptability to different soil management systems, and are rich sources of primary metabolites, vitamins and minerals; therefore, they are valuable in terms of human health and as functional food ingredients.

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**Key words:** black currant, soil management system, primary metabolites, vitamins, minerals

**Introduction.** Small berries, including black currant, are widely recognized for their nutritional quality and potential health benefits. Black currants are a good source of sugars and organic acids as important primary metabolites, vitamins, antioxidants and phenolic acids that contribute to the quality of taste and aroma [1]. Sugars and organic acids are the main soluble constituents of berries, and they have a major effect on taste, flavour and fruit ripeness, or are even used as indicators of consumer acceptance [2]. Bordonaba and Terry [3] reported that sugar and acid content and sugar to acid ratio in black currants and other fruits are essential in flavour formation. Also, black currants are an inexhaustible source of vitamins, especially vitamin C, which along with minerals make the fruit highly physiologically valuable. Major minerals and essential trace elements are very important in biological processes, play a vital role in normal growth and development, and are also involved in the prevention of some chronic diseases [4]. The different parts of black currant (root, bud, leaf, fruit) are used to effectively treat a number of diseases, such as chronic rheumatoid arthritis, respiratory problems and inflammatory disorders, and are valuable for their diuretic properties [5]. Zheng et al. [6] emphasized that biochemical composition may be affected by various factors, such as genotype, growth conditions, including environmental factors, and cultivation techniques. Numerous studies have shown that mulching orchard soils with sawdust, black plastic, bark and wood chips favours the vegetative and generative potential of black currants, and has a positive effect on the quality and chemical properties of the fruit [7]. Soil mulching facilitates the retention of soil moisture, helps control temperature fluctuations, and improves physical, chemical and biological properties of the soil, as it adds nutrients to the soil, thus ultimately enhancing the growth and yield of crops [8].

Therefore, the objective of this study was to evaluate and compare the effects of different soil management systems in black currant planting on the contents of primary metabolites, vitamins and minerals in the berries and leaves of the tested cultivars.

**Materials and methods.** The research was conducted at the Fruit Research Institute, Čačak, Western Serbia, during 2012–2016. Seven cultivars were used: ‘Ben Lomond’, ‘Ben Sarek’, ‘Ćaćanska Crna’, ‘Tsema’, ‘Titania’, ‘Tisel’ and ‘Tiben’. Three soil management systems were employed: treatment 1 – bare fallow, i.e. continuous tillage, treatment 2 – sawdust mulch, and treatment 3 – black plastic mulch. The experiment was laid out in a randomized block design (7 cultivars $\times 3$ soil management systems $\times 3$ replications), with 5 bushes per replication, giving a total of 315 black currant bushes. Fruits were sampled at full ripeness in June, while leaf samples were collected in July, at the stage of full development. Berries and leaves were selected from the inner and outer range of the bush. A total of 100 g berries and 50 leaves were sampled from 5 bushes per replication.

***Chemical analysis of the berries and leaves.*** 1. Individual invert sugars (glucose, fructose, sucrose) were determined by high-performance liquid chromatography (HPLC; Waters Breeze, Milford, USA). The detection of sugars was performed on a 2465 Waters electrochemical detector (Waters, Milford, USA). The column was CarboPac PA1 (Dionex, Sunnyvale, CA, USA) operated at 30 °C. The absorbance was measured at 210 nm and 327 nm. 2. Organic acids (citric acid, malic acid) were analyzed using a Hewlett-Packard HP1100 system equipped with a photodiode array detector (Palo, Alto, CA, USA). The column was Aminex – HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) operated at 40 °C. The absorbance was measured at 490 nm and 600 nm. 3. Minerals (K, Ca and Fe) were determined by flame atomic absorption spectrophotometry using a Varian Spectar AA 200 instrument equipped with a GTA 110 graphite furnace (Varian, USA). For each batch of samples, the absorbance of the calibration standard solution was measured. Phosphorus concentration was assessed by spectrophotometry i.e. by an MA9523-SPEKOL 211 spectrophotometer (ISKRA, Horjul, Slovenia) on the basis of the specific absorbance of light (725 nm) of the blue-coloured phospho-molybdenum complex. Upon completion, the spectrophotometer measured the absorbance at 725 nm using a calibration chart. 4. Vitamin C was assessed by a Perkin Elmer UV/VIS spectrometer (Lombda 25). The absorbance was measured at 665 nm. A high-performance liquid chromatograph (HPLC; Milford, MA, USA) fitted with a fluorescence detector was used for the analysis of vitamins A and B3. Absorption spectra were recorded at the following wavelengths: 347 nm for vitamin B3, and 295–330 nm for vitamin A.

***Statistical analysis***. The experimental data obtained during the five-year period were subjected to statistical analysis using Fisher's two-factor analysis of variance – ANOVA. Significant differences between the mean values of the tested factors and the interaction means were determined by LSD test at $p \leq 0.05$ significance levels.

**Results and discussion.** The present data demonstrate that fructose was the dominant invert sugar in all berry and leaf extracts, followed by glucose, while the amount of sucrose was very low (Tables 1 and 2). The content of fructose in the berries ranged from 134.0 to 118.3 mg $g^{-1}$, and was 4.35 times higher than in the leaves (29.9 to 28.2 mg $g^{-1}$, respectively). 'Ben Lomond' berries had the highest content of individual invert sugars, whereas the lowest values were found in 'Ben Sarek'. The leaves of the studied cultivars showed no significant differences in the content of individual invert sugars. 'Ben Lomond', 'Ben Sarek', 'Titania' and 'Ćaćanska Crna' leaves had the highest content of fructose, while 'Tisel' and 'Tiben' had higher contents of glucose and sucrose in the leaves than the other cultivars. In this study, the main organic acid was citric acid, while twice as low quantities of malic acid were found in all tested berries and leaves

Table 1

Contents of sugars and acids in berry extracts

| Cultivar/ Treatment | Fructose (mg $g^{-1}$) | Glucose (mg $g^{-1}$) | Sucrose (mg $g^{-1}$) | Citric acid (mg $g^{-1}$) | Malic acid (mg $g^{-1}$) |
|---|---|---|---|---|---|
| 'Ben Lomond' | 133.4 ± 1.44 a | 91.3 ± 1.81 a | 16.3 ± 1.16 a | 1.27 ± 0.07 c | 0.33 ± 0.03 bc |
| 'Ben Sarek' | 118.3 ± 3.48 e | 76.1 ± 2.25 e | 9.44 ± 0.54 f | 1.04 ± 0.05 e | 0.33 ± 0.03 bc |
| 'Tsema' | 122.9 ± 1.81 d | 81.2 ± 2.46 d | 11.8 ± 1.23 d | 1.18 ± 0.10 d | 0.29 ± 0.02 d |
| 'Titania' | 127.7 ± 1.82 c | 83.0 ± 2.03 c | 12.0 ± 0.98 d | 1.29 ± 0.09 c | 0.29 ± 0.02 d |
| 'Ćaćanska Crna' | 134.0 ± 2.11 a | 84.0 ± 1.25 c | 13.5 ± 1.22 b | 1.37 ± 0.08 b | 0.34 ± 0.02 ab |
| 'Tisel' | 123.8 ± 1.64 d | 80.7 ± 2.36 d | 11.2 ± 1.11 e | 1.56 ± 0.09 a | 0.39 ± 0.03 a |
| 'Tiben' | 129.6 ± 1.33 b | 86.0 ± 1.30 b | 12.9 ± 1.24 c | 1.33 ± 0.07 bc | 0.30 ± 0.03 d |
| bare fallow | 126.3 ± 1.44 b | 82.8 ± 1.40 b | 12.3 ± 0.75 b | 1.21 ± 0.05 c | 0.32 ± 0.02 ab |
| sawdust | 128.6 ± 1.39 a | 83.9 ± 1.30 a | 12.2 ± 0.73 b | 1.35 ± 0.05 a | 0.34 ± 0.02 a |
| black plastic | 126.4 ± 1.47 b | 82.9 ± 1.36 b | 12.9 ± 0.73 a | 1.30 ± 0.05 b | 0.31 ± 0.02 b |
| ANOVA | | | | | |
| Cultivar (A) | ** | ** | ** | ** | ** |
| Treatment (B) | ** | ** | ** | ** | * |
| A × B | ns | ns | ns | ns | ns |

Means followed by different letters within the cultivar and treatment columns are significantly different at $p \leq 0.05$ according to LSD test and ANOVA ($F$-test) results

(Tables 1 and 2, respectively). The highest concentrations of citric acid and malic acid were recorded in the berries of 'Tisel', whereas 'Tsema' had the highest levels of these acids in the leaves. Mladin et al. [9] reported high levels of organic acids in black currant cultivars. The soil management systems had a significant effect on the tested parameters. In this study, the berries of black currants grown on sawdust mulch had the highest levels of fructose, glucose and organic acids, and those on black plastic mulch had a high content of sucrose. In the leaves, the levels of individual invert sugars and organic acids were highest in currants grown on black plastic. It is most likely that the higher temperature and moisture of the soil under plastic mulch favoured the synthesis of individual invert sugars and organic acids in the leaves, as well as the level of sucrose in the berries, whereas the smaller temperature fluctuations and higher soil moisture under sawdust mulch promoted the synthesis of fructose, glucose and organic acids in the berries. Kasperbauer et al. [10] mentioned that red and far-red light reflected from mulch is captured by phytochrome, which triggers a series of chemical modifications in fruits that alter sugar and organic acid contents, and contributes to improved sweetness and flavour of the fruit from plants grown on mulch. Decoteau et al. [11] affirmed that plants grown on different types of plastic mulches respond to even the small

Table 2

Contents of sugars and acids in leaf extracts

| Cultivar/ Treatment | Fructose (mg $g^{-1}$) | Glucose (mg $g^{-1}$) | Sucrose (mg $g^{-1}$) | Citric acid (mg $g^{-1}$) | Malic acid (mg $g^{-1}$) |
|---|---|---|---|---|---|
| 'Ben Lomond' | 29.9 ± 0.66 a | 20.2 ± 0.57 c | 2.44 ± 0.04 b | 0.22 ± 0.007 c | 0.014 ± 0.0005 b |
| 'Ben Sarek' | 29.9 ± 0.66 a | 19.8 ± 0.56 d | 2.45 ± 0.04 b | 0.22 ± 0.007 c | 0.014 ± 0.0006 b |
| 'Tsema' | 28.5 ± 0.52 b | 22.6 ± 0.50 b | 2.59 ± 0.04 a | 0.26 ± 0.006 a | 0.018 ± 0.0002 a |
| 'Titania' | 29.8 ± 0.68 a | 19.8 ± 0.57 d | 2.44 ± 0.03 b | 0.22 ± 0.008 c | 0.014 ± 0.0006 b |
| 'Ćaćanska Crna' | 29.9 ± 0.66 a | 19.8 ± 0.58 d | 2.43 ± 0.04 b | 0.23 ± 0.008 b | 0.012 ± 0.0004 c |
| 'Tisel' | 28.2 ± 0.56 b | 23.2 ± 0.55 a | 2.65 ± 0.05 a | 0.24 ± 0.007 b | 0.015 ± 0.0002 b |
| 'Tiben' | 28.3 ± 0.57 b | 23.1 ± 0.56 a | 2.65 ± 0.05 a | 0.23 ± 0.006 b | 0.015 ± 0.0002 b |
| bare fallow | 29.2 ± 0.12 b | 17.6 ± 0.17 c | 2.34 ± 0.02 c | 0.23 ± 0.004 b | 0.013 ± 0.0002 c |
| sawdust | 26.8 ± 0.44 c | 22.0 ± 0.34 b | 2.58 ± 0.03 b | 0.22 ± 0.005 c | 0.015 ± 0.0001 b |
| black plastic | 31.6 ± 0.33 a | 24.0 ± 0.09 a | 2.64 ± 0.02 a | 0.24 ± 0.005 a | 0.017 ± 0.0001 a |
| ANOVA | | | | | |
| Cultivar (A) | ** | ** | ** | ** | ** |
| Treatment (B) | ** | ** | ** | ** | ** |
| A × B | ns | ns | ns | ns | ns |

Means followed by different letters within the cultivar and treatment columns are significantly different at $p \leq 0.05$ according to LSD test and ANOVA ($F$-test) results

changes in ambient light induced by mulch colour. The highest values of fruit size, yield, and ascorbic acid and anthocyanin levels, as observed under black polythene mulch, indicate a greater role of elevated soil temperature as a catalyst for root activities, including the uptake of water and nutrients, which is ultimately reflected in higher yield and better quality of the fruit [12].

The vitamin contents of berry and leaf extracts are presented in Tables 3 and 4, respectively. In this study, significant differences were observed in the content of vitamins between berries and leaves.

The main vitamins in black currant berries and leaves were vitamin C (223.9 and 39.3 mg 100 $g^{-1}$, respectively), followed by vitamin B3 (40.5 and 14.4 mg 100 $g^{-1}$, respectively) and vitamin A (19.1 and 6.35 mg 100 $g^{-1}$, respectively). Berry extracts contained 2.81 to 6.23 times more vitamins than leaf extracts. 'Ćaćanska Crna' had the highest content of vitamin C, whereas 'Ben Lomond' had higher amounts of vitamins A and B3. In the leaves, the highest contents of vitamins were found in 'Ben Sarek', whereas the other cultivars exhibited variability in the studied parameters. The present results are comparable to those of Nes et al. [13], but they are not in agreement with Raudsepp et al. [5]

T a b l e 3

Contents of vitamins and minerals in berry extracts

| Cultivar/ Treatment | Vitamin C (mg 100 $g^{-1}$) | Vitamin A (mg 100 $g^{-1}$) | Vitamin B3 (mg 100 $g^{-1}$) | K (mg 100 $g^{-1}$) | P (mg 100 $g^{-1}$) | Ca (mg 100 $g^{-1}$) | Fe (mg 100 $g^{-1}$) |
|---|---|---|---|---|---|---|---|
| ‘Ben Lomond’ | 215.4 ± 2.48 c | 19.1 ± 0.37 a | 40.5 ± 0.77 a | 330.0 ± 1.91 a | 189.5 ± 0.79 c | 30.1 ± 0.38 cd | 5.74 ± 0.32 a |
| ‘Ben Sarek’ | 209.9 ± 1.82 d | 17.8 ± 0.42 e | 39.5 ± 0.43 e | 328.4 ± 2.11 c | 188.7 ± 0.56 d | 34.4 ± 0.83 a | 5.58 ± 0.33 ab |
| ‘Tsema’ | 219.3 ± 2.99 b | 17.3 ± 0.37 f | 39.9 ± 0.72 d | 327.0 ± 2.39 d | 188.9 ± 0.79 d | 30.1 ± 0.39cd | 5.02 ± 0.24 d |
| ‘Titania’ | 209.0 ± 2.21 de | 18.4 ± 0.45 c | 38.6 ± 0.27 f | 329.6 ± 2.44 ab | 190.1 ± 0.58 b | 29.9 ± 0.42 d | 5.23 ± 0.30 c |
| ‘Ćaćanska Crna’ | 223.9 ± 3.03 a | 18.9 ± 0.42 b | 40.4 ± 0.67ab | 327.6 ± 2.44 d | 190.3 ± 0.65 b | 31.8 ± 0.58 b | 5.72 ± 0.34 a |
| ‘Tisel’ | 207.9 ± 2.37 e | 18.1 ± 0.35 d | 40.2 ± 0.73 bc | 325.2 ± 2.23 e | 190.8 ± 0.71 a | 29.9 ± 0.34 d | 5.51 ± 0.29 b |
| ‘Tiben’ | 209.0 ± 2.42 de | 18.4 ± 0.42 c | 40.0 ± 0.72 cd | 329.1 ± 2.15 bc | 190.9 ± 0.73 a | 30.3 ± 0.42 c | 5.51 ± 0.30 b |
| bare fallow | 213.0 ± 1.64 b | 18.3 ± 0.26 b | 40.0 ± 0.42 a | 327.7 ± 1.44 b | 189.8 ± 0.45 | 30.9 ± 0.36 b | 5.62 ± 0.20 a |
| sawdust | 214.1 ± 1.81 a | 18.3 ± 0.27 b | 39.8 ± 0.43 b | 327.4 ± 1.45 b | 189.8 ± 0.45 | 31.1± 0.35 a | 5.47 ± 0.20 b |
| black plastic | 213.3 ± 1.71 b | 18.1 ± 0.27 a | 39.8 ± 0.41 b | 329.3 ± 1.51 a | 190.0 ± 0.46 | 30.8 ± 0.37 b | 5.34 ± 0.19 c |
| ANOVA | | | | | | | |
| Cultivar (A) | ** | ** | ** | ** | ** | ** | ** |
| Treatment (B) | ** | ** | ** | ** | ** | ** | ** |
| A × B | ns | ns | ns | ns | ns | ns | ns |

Means followed by different letters within the cultivar and treatment columns are significantly different at $p \leq 0.05$ according to LSD test and ANOVA ($F$-test) results

Table 4

Contents of vitamins and minerals in leaf extracts

| Cultivar/ Treatment | Vitamin C (mg 100 $g^{-1}$) | Vitamin A (mg 100 $g^{-1}$) | Vitamin B3 (mg 100 $g^{-1}$) | K (mg 100 $g^{-1}$) | P (mg 100 $g^{-1}$) | Ca (mg 100 $g^{-1}$) | Fe (mg 100 $g^{-1}$) |
|---|---|---|---|---|---|---|---|
| ‘Ben Lomond’ | 38.6 ± 0.17 c | 6.35 ± 0.09 a | 14.4 ± 0.08 a | 20.4 ± 0.04 a | 24.1 ± 0.10 a | 2.58 ± 0.03 a | 0.51 ± 0.02 bc |
| ‘Ben Sarek’ | 39.3 ± 0.08 a | 6.35 ± 0.09 a | 14.4 ± 0.08 a | 20.3 ± 0.03 b | 24.1 ± 0.10 a | 2.57 ± 0.03 a | 0.51 ± 0.02 bc |
| ‘Tsema’ | 38.4 ± 0.16 d | 5.99 ± 0.08 b | 14.3 ± 0.12 b | 20.3 ± 0.06 b | 23.4 ± 0.20 b | 2.58 ± 0.03 a | 0.52 ± 0.02 b |
| ‘Titania’ | 38.6 ± 0.17 c | 6.35 ± 0.09 a | 14.3 ± 0.08 b | 20.3 ± 0.03 b | 24.1 ± 0.10 a | 2.58 ± 0.03 a | 0.50 ± 0.02 c |
| ‘Ćaćanska Crna’ | 38.6 ± 0.17 c | 6.35 ± 0.09 a | 14.4 ± 0.08 a | 20.3 ± 0.03 b | 24.1 ± 0.10 a | 2.57 ± 0.02 a | 0.51 ± 0.02 bc |
| ‘Tisel’ | 38.7 ± 0.11 b | 6.13 ± 0.08 b | 13.9 ± 0.11 c | 20.2 ± 0.06 c | 22.8 ± 0.19 c | 2.51 ± 0.02 b | 0.56 ± 0.01 a |
| ‘Tiben’ | 38.7 ± 0.11 b | 6.14 ± 0.08 b | 13.9 ± 0.11 c | 20.2 ± 0.06 c | 22.8 ± 0.19 c | 2.51 ± 0.02 b | 0.56 ± 0.01 a |
| bare fallow | 38.5 ± 0.03 b | 6.19 ± 0.07 b | 13.6 ± 0.06 c | 20.4 ± 0.03 a | 23.5 ± 0.14 b | 2.53 ± 0.01 b | 0.51 ± 0.02 b |
| sawdust | 39.2 ± 0.06 a | 6.39 ± 0.03 a | 14.6 ± 0.02 a | 20.2 ± 0.03 b | 23.5 ± 0.08 b | 2.51 ± 0.01 b | 0.56 ± 0.01 a |
| black plastic | 38.3 ± 0.13 c | 6.13 ± 0.05 b | 14.4 ± 0.05 b | 20.2 ± 0.02 b | 23.9 ± 0.13 a | 2.64 ± 0.02 a | 0.49 ± 0.02 c |
| ANOVA | | | | | | | |
| Cultivar (A) | ** | ** | ** | ** | ** | ** | ** |
| Treatment (B) | ** | ** | ** | ** | ** | ** | ** |
| A × B | ns | ns | ns | ns | ns | ns | ns |

Means followed by different letters within the cultivar and treatment columns are significantly different at $p \leq 0.05$ according to LSD test and ANOVA ($F$-test) results and Mladin et al. [9] who recorded lower values for vitamin C. Tabart et al. [14] reported a higher content of ascorbic acid in black currant berries than in the leaves and buds. This study demonstrated large differences in the amount of vitamins depending on the soil management system. Black currants grown on sawdust mulch had the highest level of vitamin C in the berries, and the highest values for vitamin C, A and B3 in the leaves. In the berries, vitamin A content was higher under black plastic mulch, as opposed to vitamin B3, which had higher values under bare fallow. The results presented by Ochmian et al. [15] indicated that mulching with sawdust increased the vitamin C content, in contrast to Nestby [16], who found an increase in vitamin C content in strawberries under polyethylene mulch. Helaly et al. [17] reported that the improvement in vitamin C content under polyethylene mulch may be due to its effect in promoting plant growth and metabolism, thus enhancing the chemical composition.

The average contents of minerals in the berries and leaves of the tested black currant cultivars are presented in Tables 3 and 4, respectively. In the present study, 'Ben Sarek' had the highest content of Ca, while 'Ben Lomond' had higher levels of K and Fe. 'Tisel' and 'Tiben' outperformed the other cultivars in P content. As for the concentration in leaves, the cultivars exhibited variability in the studied minerals. Black currants grown on black plastic mulch gave the highest levels of Ca and P in the leaves and the highest content of K in the berries possibly due to the high temperature and moisture of the soil, and those on sawdust mulch had a high content of Fe in the leaves most likely due to small temperature fluctuations and high soil moisture. Moderate soil temperature and moisture conditions under bare fallow treatment promoted the synthesis of K in the leaves and Fe in the berries. Compared to the present experiment, Perkins-Veazie and Collins [18] reported higher values for K and Ca, but significantly lower values for P and Fe, whereas Nour et al. [19] obtained comparable levels of Ca, and lower amounts of K and Fe.

Generally, black currants are an exceptionally rich source of sugars, acids, vitamins and minerals, and an interesting nutritional alternative. The highest values for primary metabolites, vitamins and minerals in berry and leaf extracts were achieved by currants grown under sawdust and black plastic mulch. Berries and leaves of black currants could be of interest to industrial applications in healthcare, and can be used as a new source of natural foods.

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